Gestion des bio-agresseurs et réduction des pesticides en culture de laitue sous abris froids : apports croisés d'expérimentations factorielles et systémiques

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Résumé

La réduction de la dépendance aux pesticides des systèmes de culture de laitue constitue un objectif à relativement court terme, partagé par les pouvoirs publics et les acteurs de la filière de production. Atteindre cet objectif suppose de disposer de moyens techniques permettant de substituer aux pesticides d'autres méthodes limitant les dégâts de bio-agresseurs de la laitue. Ces techniques peuvent être destinées à minimiser l'entrée d'inoculum dans les parcelles, à limiter la propagation des bio-agresseurs, à diminuer la sensibilité des plantes, ou à éradiquer les bio-agresseurs présents sur les cultures. L'évolution des systèmes de culture repose sur la démonstration de l'efficacité, en termes de protection des plantes, de ces techniques alternatives aux pesticides lorsqu'elles sont intégrées et combinées dans les itinéraires techniques. Elle dépend aussi de l'impact socio-économique et environnemental des itinéraires techniques alternatifs, dans un contexte commercial et réglementaire exigeant. L'objet de cette thèse a été de développer des stratégies alternatives de gestion des bio-agresseurs de la laitue d'hiver cultivée sous abri froid, plus économes en pesticides, et d'évaluer leurs performances agronomiques, économiques et environnementales, garantes de la durabilité des systèmes de production.

Sur la base des techniques déjà disponibles, deux stratégies alternatives, nommées stratégie intermédiaire et stratégie bas-intrant ont été conçues, et testées dans deux exploitations agricoles et un domaine expérimental de l'INRA pendant deux hivers, en comparaison avec une stratégie conventionnelle, représentative des pratiques actuelles. Les stratégies intermédiaire et bas-intrant ont permis de réduire de 32% et 48% l'usage des pesticides, respectivement, et ont été suffisamment efficaces pour obtenir une qualité visuelle et des rendements équivalents à la stratégie conventionnelle. Le bénéfice environnemental de leur mise en œuvre a également été démontré. La mise en place de ces stratégies a cependant entrainé un surcoût de production, essentiellement lié à la lutte biologique, d'environ 10 à 13%.

Parallèlement, deux orientations techniques originales, dont l'efficacité n'était pas caractérisée, ont été explorées. Une première série d'essais expérimentaux a porté sur l'influence du génotype de l'hôte et de l'environnement de la plante sur sa sensibilité à deux champignons pathogènes d'importance majeure, *Botrytis cinerea* et *Sclerotinia sclerotiorum*. Il a été montré que le choix d'un génotype moins sensible couplé à une optimisation du rapport fructose : saccharose de la plante diminuait les symptômes observés après inoculation. Une deuxième démarche expérimentale, portant sur la lutte biologique contre le puceron *Nasonovia ribisnigri*, a révélé l'incapacité de parasitoïdes du genre *Aphidius* à contrôler les pucerons en fin de culture, lorsque la structure du couvert végétal devient trop complexe.

En plus d'inscrire ces travaux de recherche dans un processus d'amélioration continue de l'efficacité et des performances des stratégies alternatives aux pesticides, la démarche scientifique utilisée, articulant approche analytique et approche intégrative, a permis d'étudier des techniques ayant potentiellement un impact sur plusieurs bio-agresseurs, telles que la réduction de la fertilisation azotée ou l'optimisation de l'espacement entre les laitues, qui pourraient permettre une meilleure gestion des pathogènes responsables de la pourriture du collet et des pucerons.

Abstract

The reduction of pesticide reliance in lettuce cropping systems is a short term objective shared by public authorities and by the stakeholders of lettuce market. Reaching this goal implies the substitution of pesticides by others techniques which may limit pest and pathogen damage. These techniques can be intended to mitigate pest and pathogen invasion or propagation, to increase plant defenses or to remove pests and pathogens from the crop. The improvement of current cropping systems relies on i) the demonstration of the ability of alternative techniques to manage diseases and pests when they are combined and integrated during the crop cycle; ii) the assessment of the socio-economic and environmental impacts of alternative strategies in a stringent commercial and regulatory context. The objective of this thesis was to design alternative strategies for pest and pathogen management of lettuce grown in winter under shelter, with fewer pesticide applications, and to assess their agronomic, economic and environmental performances so as to ensure the sustainability of production.

Based on currently available techniques, two alternative strategies, called intermediate and low-input, were designed, and tested in two farms and an INRA experimental station during two winters and compared to a conventional strategy representing current practices. The alternative strategies enabled to reduce pesticide applications by 32% and 48% respectively. They were efficient enough to obtain similar yield and quality as compared to the conventional strategy. The environmental benefits of their implementation were also demonstrated. However, these strategies required a 10-13% extra production costs, almost exclusively due to biological control products.

In parallel, two original technical orientations, which efficacy had not been previously described, were examined. In a first set of experiments, the impact of lettuce genotype and growth conditions on plant susceptibility to two major pathogens, *Botrytis cinerea* and *Sclerotinia sclerotiorum*, was investigated. The use of a genotype displaying low susceptibility to these fungi, associated with an optimization of the ratio of fructose : sucrose in plants, appeared to reduce the symptoms after inoculation. A second experimental approach, dealing with biological control against the aphid *Nasonovia ribisnigri*, highlighted the inability of *Aphidius* parasitoids to control aphid populations at the end of the crop cycle, when the canopy structure becomes too complex.

In addition to a contribution to cropping systems improvement, the scientific method used, combining analytical and integrative approaches enabled us to highlight the effect of techniques acting on several pests and/or pathogens, such as the reduction of nitrogen fertilization or the optimization of plant spacing, which could improve the management of fungi causing basal rot as well as aphids.

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Introduction

Chapitre 1

Contexte et Problématique

1.1 Contexte

La plupart des systèmes de production agricoles sont encore largement dépendants des produits phytosanitaires (Wilson and Tisdell, 2001). Introduit massivement dans les itinéraires techniques au cours du siècle passé, les pesticides (insecticides, fongicides et herbicides) ont contribué à améliorer et à stabiliser les rendements agricoles, et à augmenter la qualité visuelle des produits de récoltes, rendus indemnes de dégâts de bio-agresseurs. Dans les productions de fruits et légumes notamment, l'absence de dégâts biotiques est devenue la norme, y compris du point de vue des consommateurs. Cependant, les effets délétères des produits phytosanitaires de synthèse ont progressivement été mis en évidence, en particulier leur rémanence dans l'environnement et leur toxicité sur les organismes non-cibles, ainsi que les risques pour la santé humaine. D'un point de vue réglementaire, les produits à large spectre les plus nocifs ont été progressivement remplacés par des molécules plus spécifiques, et les règles d'usage ont été durcies, avec notamment la mise en place de normes précisant les teneurs maximales en résidus dans les produits de récolte. Cependant, dans de nombreuses régions du monde, l'acceptabilité sociale des pesticides diminue constamment et la réduction de la dépendance des systèmes de production aux pesticides constitue aujourd'hui un enjeu majeur pour l'agriculture.

En 2009, la commission européenne a instauré une directive (2009/128/CE - European Parliement, 2009) visant à réduire l'usage des pesticides dans les pays de l'Union Européenne. La France, anticipant la parution de cette directive, a mis en place le plan Ecophyto (2008), qui a pour objectif de réduire de 50%, « si possible », l'usage des pesticides d'ici à 2018. Les productions de fruits et légumes, dans lesquelles les produits de récolte arrivent pour l'essentiel sans transformation auprès des consommateurs finaux, sont fortement consommatrices de produits phytosanitaires : les fruits et légumes comptent pour 1.6% de la SAU en France, mais pour 9.9% du volume des pesticides utilisés (source INRA, expertise Ecophyto R&D). Pour rester compétitifs, les agriculteurs doivent produire des fruits et légumes satisfaisant des exigences qui peuvent paraître contradictoires : un aspect visuel irréprochable et des teneurs en résidus de pesticides de plus en plus faibles. La filière laitue constitue un bon exemple de cette dualité (Palumbo, 2009).

La laitue est une culture économiquement importante pour la France. Avec près de 250 000 tonnes produit par an, la France est le $3^{ème}$ producteur Européen de laitues derrière l'Espagne et l'Italie (Agreste, 2014). La salade (laitue et chicorée) est le deuxième légume le plus exporté après la tomate, principalement vers le nord de l'Europe (Allemagne, Grande-Bretagne, etc.). Les exportations représentent 21.4% de la production Française (FranceAgriMer, 2014), et les exigences des marchés d'export sur la qualité des produits peuvent être encore plus drastiques que celles du marché intérieur. La laitue est un légume-feuilles dont la partie aérienne est consommée majoritairement crue et presque dans son intégralité. Les seuils de tolérance pour la présence de « corps étrangers » et de dégâts d'origine biotique ou abiotique sont très bas. La présence de résidus de pesticides et de nitrate dans les laitues est également contrôlée et les exigences de certains acheteurs (usines pour la $4^{ème}$ gamme et grandes et moyennes surfaces - GMS), en ce qui concerne ces teneurs, sont souvent plus strictes que la législation. La réduction des pesticides

est en conséquence un objectif partagé par les pouvoirs publics et les acteurs de la filière laitue. Les objectifs des parties prenantes convergent également en ce qui concerne la vitesse d'évolution des pratiques, avec des résultats attendus à relativement court terme. Fortes de ce constat, la société Green Produce, dont l'activité réside dans l'approvisionnement d'usines de découpe de légumes ($4^{ème}$ gamme) situées dans toute l'Europe, et l'unité PSH de l'INRA d'Avignon se sont associées pour mettre en œuvre des recherches visant à réduire l'usage des pesticides en culture de laitue, au moyen de nouveaux itinéraires techniques qui n'affecteraient pas la compétitivité des exploitations, c'est-à-dire sans pertes de rendement ou de qualité des produits commercialisés. Le travail de thèse présenté ici, soutenu et financé par le plan Ecophyto et l'Agence Nationale de la Recherche et de la Technologie, restitue les premiers résultats de ces recherches.

1.2 Problématique et objectifs de la thèse

La laitue est une plante annuelle de la famille des *Asteraceae*, cultivée toute l'année en plein champ ou sous abris (tunnels ou multi-chapelles) (Figure 1.1). Les principaux types horticulturaux cultivés en Europe sont les laitues beurre, les batavias, les laitues à couper (ne formant pas une pomme) et les romaines (Thicoipe, 1997) (Figure 1.2). Les bio-agresseurs susceptibles de causer des dégâts sur les cultures de laitue sont nombreux, et d'importance inégale en fonction des saisons. Il peut s'agir de champignons phytopathogènes (*Bremia lactucae*, *Botrytis cinerea*, *Sclerotinia* spp., etc.) de ravageurs (pucerons, noctuelles, thrips, etc .), de virus ou encore de bactéries. La gestion de ces bio-agresseurs en agriculture conventionnelle se fait majoritairement par des applications de pesticides. Les délais avant récolte (DAR) légaux des produits phytopharmaceutiques sont souvent longs au regard de la durée du cycle de culture de la laitue. Les DAR sont généralement compris entre 14 et 28 jours pour les fongicides et entre 7 et 14 jours pour les insecticides. En conséquence, les pesticides, et surtout les fongicides, sont principalement appliqués de manière préventive.

Nous pouvons schématiquement distinguer deux types d'approches pouvant être utilisées dans des démarches visant à réduire l'usage des pesticides. Un premier type d'approche, analytique, vise à explorer la biologie des bio-agresseurs et/ou des plantes hôtes, et à développer des techniques ou technologies pour maîtriser leurs interactions, au bénéfice de la plante. La démonstration de l'efficacité de ces techniques et technologies repose souvent sur des expérimentations mono ou bi-factorielles. Le second type d'approche repose sur des études intégratives visant à combiner plusieurs techniques alternatives aux pesticides, dont l'efficacité est partielle, et à étudier l'impact global sur les populations de bio-agresseurs, sans chercher à évaluer l'effet particulier de chaque technique. Cette seconde approche repose sur l'hypothèse que les techniques à efficacité partielle peuvent présenter des complémentarités, voire des synergies, permettant de réduire significativement la pression des maladies et des ravageurs.

L'expertise Ecophyto R&D (Brismontier, 2009), dans sa partie consacrée aux cultures légumières, a suggéré que la réduction des pesticides pourrait induire des pertes de rendement importantes (de l'ordre de 25%) et une augmentation des coûts de production. De fait, les productions de laitue s'affranchissant totalement des pesticides de synthèse, c'est-à-dire les productions en agriculture biologique, se distinguent par des prix de vente plus élevés, de l'ordre du double de ceux observés en agriculture conventionnelle. Il n'y a pas en France, à notre connaissance, d'étude sur les impacts agronomiques, économiques et environnementaux d'une réduction de l'usage des pesticides en culture de laitue. L'étude de la littérature scientifique internationale ne permet pas non plus d'apporter une réponse. L'objectif de cette étude est en conséquence de proposer et de tester des stratégies durables de protection de la laitue, satisfaisant les objectifs de réduction de pesticides fixés par les industriels (quantité de résidus de pesticides limitée) et les pouvoirs publics (objectif Ecophyto 2018 : -50%), tout en maintenant la qualité visuelle des produits. De nombreuses combinaisons associant des variantes de type horticultural, de structure de production et de marché existent et ne peuvent pas toutes être explorées dans l'espace d'un travail de thèse. La Batavia (le type horticultural le plus produit en France) cultivée sous abri froid et destinée au marché de $4^{ème}$ gamme, qui est le plus exigeant concernant la qualité des laitues (présence de résidus, de corps étrangers, qualité visuelle), a été utilisée comme objet d'étude.

FIGURE 1.1: Différentes infrastructures de production de laitues. **a** : Plein champ ; **b** : Tunnel froid ; **c** : Multi-chapelle.

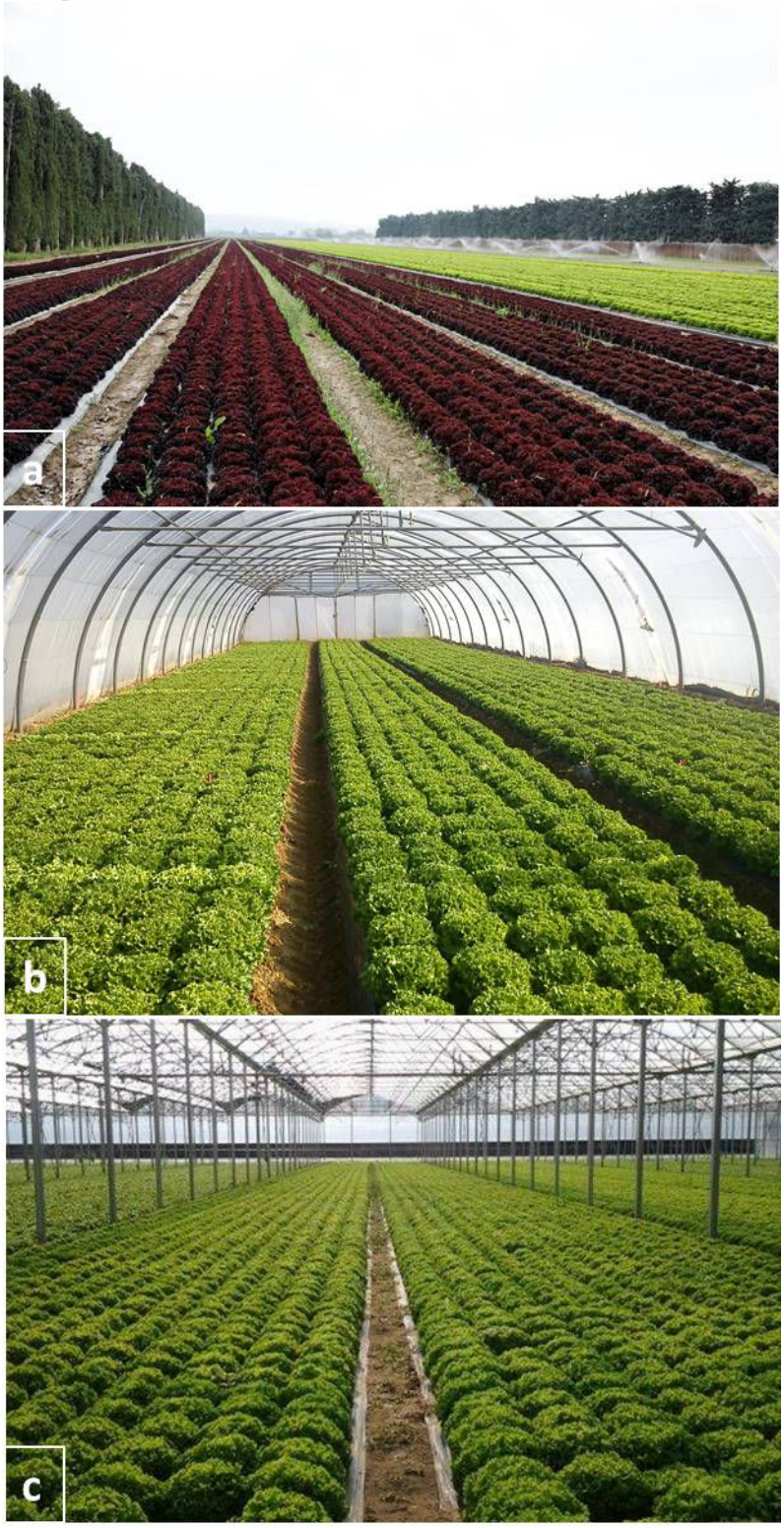
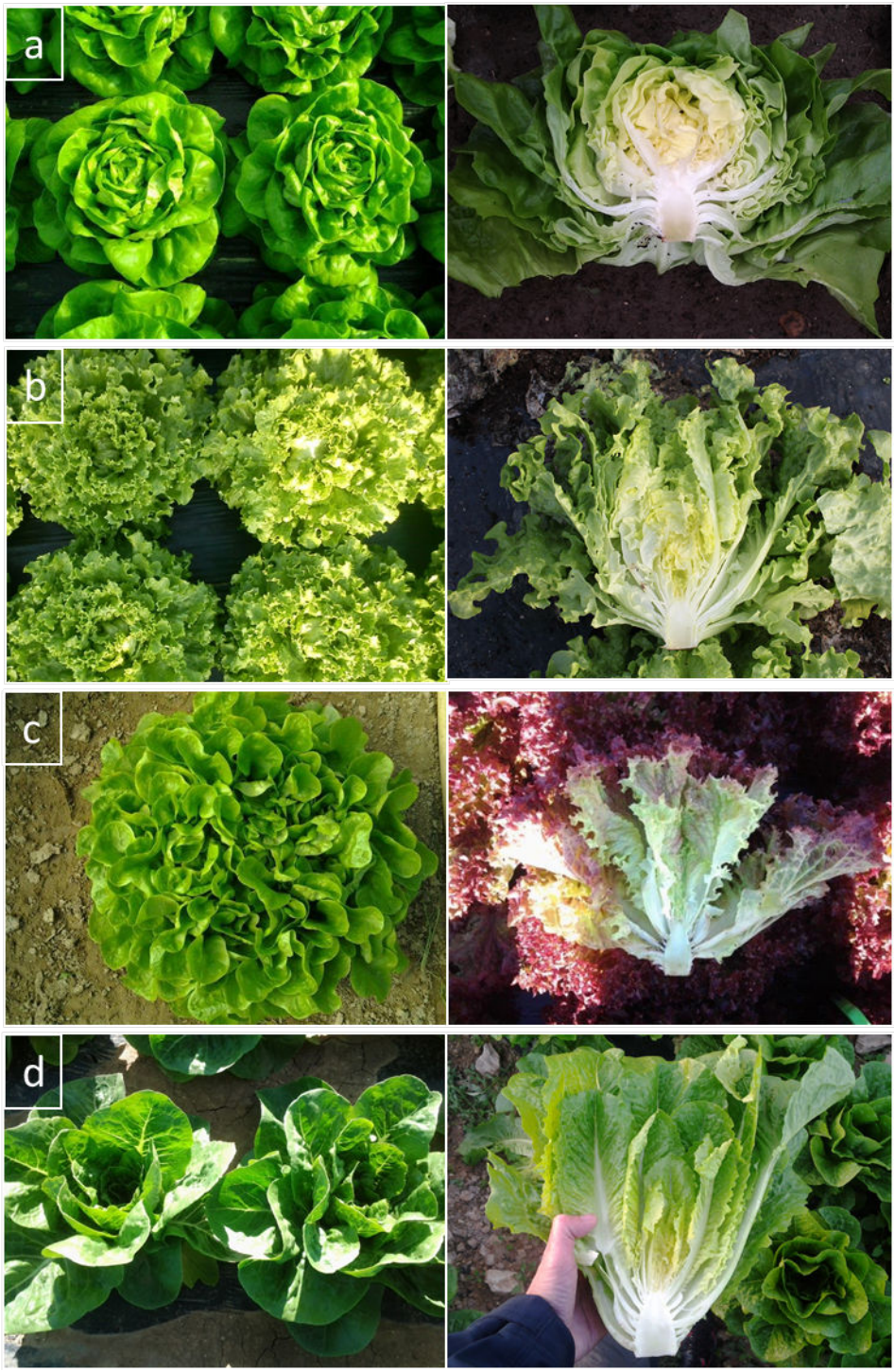


FIGURE 1.2: Principaux types horticulturaux cultivés en Europe, plante entière et coupe longitudinale. **a** : Laitue beurre ; **b** : Batavia ; **c** : Laitues à couper ; **d** : Romaines.



Ce travail, dont la démarche s'inscrit dans les deux types d'approches évoquées ci-dessus, s'est développé à partir de deux questions initiales : Disposons nous d'une réserve suffisante de techniques alternatives aux pesticides dont l'efficacité permettrait de limiter le recours à la lutte chimique, sans pour autant s'en affranchir totalement ? L'introduction et la combinaison de ces techniques dans des stratégies alternatives de gestion des bio-agresseurs permettrait-elle d'atteindre les objectifs de réduction de pesticides fixés par le plan Ecophyto et les acteurs de la filière ? Le chapitre 2 propose une revue de la littérature scientifique. Cette synthèse identifie les bio-agresseurs de la laitue, répertorie les techniques alternatives aux pesticides disponibles, et évalue les contraintes et les outils pour les assembler au sein de stratégies de gestion des bio-agresseurs. Deux constatations fortes émanent de ce diagnostic et ont permis de définir les objectifs de la thèse :

1. Bien que de nombreuses avancées sur les techniques alternatives aux pesticides aient été réalisées depuis plusieurs décennies, certaines informations sur les leviers génétiques, environnementaux et culturaux du contrôle des bio-agresseurs font défaut et peuvent limiter les possibilités de mise en œuvre pratique des techniques. En conséquence, des travaux de recherche ont été menés dans le cadre de cette étude, visant à apporter de nouvelles informations sur :
   — les niveaux de résistance génétique et les conditions environnementales favorables à la défense des plantes face à deux champignons nécrotrophes, *Botrytis cinerea* et *Sclerotinia sclerotiorum*, qui provoquent des dégâts importants en culture de laitue (Chapitre 3) ;
   — Les effets de la fertilisation azotée sur le développement des populations de pucerons et des niveaux trophiques supérieurs (Chapitre 4).
2. Des études expérimentales sont nécessaires pour évaluer les risques liés à une diminution d'usage de produits phytosanitaires. Une approche intégrative a été mise en œuvre, consistant à concevoir et mettre en place des stratégies alternatives de protection de la laitue et à évaluer l'efficacité et les performances économiques et environnementales de ces stratégies. Les résultats de cette démarche sont présentés dans la troisième partie de ce document (Chapitres 5 et 6).

Finalement, les apports croisés des approches analytique et intégrative pour la gestion des bio-agresseurs de la laitue (Figure 1.3) sont discutés dans la quatrième et dernière partie.

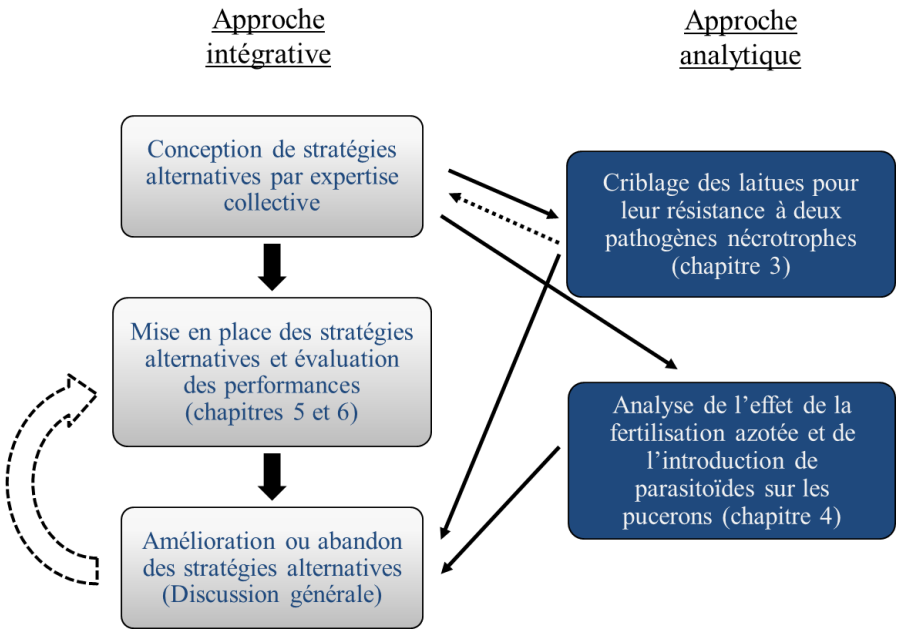


FIGURE 1.3: Démarche scientifique utilisée pour analyser les techniques et combinaisons de techniques alternatives aux pesticides.

Chapitre 2

Lettuce cropping with less pesticides. A review [1]

Virginie Barrière . François Lecompte . Philippe C. Nicot . Brigitte Maisonneuve . Marc Tchamitchian . Françoise Lescourret

Abstract

Agricultural intensification has increased crop productivity but decreased agroecosystem services. Agricultural intensification is occurring notably for horticultural crops such as lettuce. In conventional agriculture, lettuce protection is achieved mostly by preventive applications of pesticides with about eight treatments for a 60–90-day-long cycle in the Mediterranean region. However, new sustainable control strategies are needed due to pesticide impact on environment and human health, emerging pesticide resistance, and stricter policies on levels of pesticide residues in food. Here, we review knowledge and methods allowing to grow lettuce with less pesticides. Advances shown are based on pest ecology and pathogen control by the agroecosystem. The major points are as follows : (1) pest and pathogen community composition depends partly on climatic conditions. The identification of pests and pathogens that can threaten the crop is the first step to design innovative lettuce cropping systems less dependent on pesticides. (2) The numerous alternative techniques currently available should be combined to control lettuce pests and pathogens. The effects of alternative techniques on nontarget organisms including non-target pests are poorly known so far. (3) Designing sustainable systems requires taking into account ecological interactions and suitability of different management techniques of low impact.

Keywords

Lactuca sativa . Pest and disease management . Agroecosystem services . Pesticides . Alternative techniques

Introduction

Since the 1960s, agricultural intensification characterized both by the simplification and the artificiality of cultivated areas has led to a sharp increase in productivity (Stoate et al. 2009 ; Matson et al. 1997). In return, intensification has also brought many negative externalities (Stoate et al. 2001) including plant diversity losses at different scales, ranging from field-wide to region-wide effects (Mediene et al. 2011). It is now recognized that biodiversity, more precisely functional diversity, plays a key role in the provision of ecological services by agroecosystems, although this

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role is still poorly understood (Mace et al. 2012). Agricultural intensification has resulted in the loss of some ecosystem services that have been replaced by cultural practices and external inputs (Matson et al. 1997 ; Altieri 1999). These inputs have, in turn, impeded other services, as exemplified by Sanchez-Moreno and Ferris (2007), who showed that tillage, fertilizer, and herbicide inputs could hamper predation and soilborne pest regulation services by disrupting high trophic levels of soil food webs. Consequently, intensively managed agrosystems have become heavily dependent on these compensatory practices. Among agricultural practices that have supplanted ecosystem services, inputs of pesticides are probably the most criticized. Pesticides have gradually come to be used regularly as a performance insurance, rather than occasionally as a curative means to control pest and pathogen outbreaks (Lamine et al. 2010).

This practice has greatly replaced and reduced natural pest regulation, which is promoted by functional diversity within food webs (Altieri 1999 ; Moonen and Barberi 2008), thus decreasing the sustainability (Lewis et al. 1997). Agricultural intensification outcomes are particularly noticeable in horticultural crops such as lettuce in the Mediterranean region. Spain, Italy, and France are the three largest European producers, producing respectively 35 %, 21 %, and 13 % of the lettuce tonnage in Europe in 2010 (Eurostat 2012). In this region, the use of genetic breeding and external inputs has allowed lettuce cultivation in open fields or inside greenhouses almost all year long (Figure 1.1).

Different types of lettuce are grown for the fresh or processed markets. The main types are crisphead, butterhead, looseleaf (e.g., batavia, oak leaf lettuce), and romaine (Lebeda et al. 2007 ; Mou 2008). In France, the types the most produced are batavia (37 %) and oak leaf (31 %), while in Spain it is crisphead (Maisonneuve and Blancard 2011). In intensively managed cropping systems, lettuce is grown on specialized farms as a monoculture or sometimes within crop rotations which typically include Cucurbitaceae and Solanaceae. Nutrient recycling and soil aeration are replaced by fertilizer inputs and by plowing, respectively. Pest and disease control is achieved with pesticides, often in a systematic and preventative way. Similarly to other horticultural crops, the visual quality of fresh market lettuce is generally expected to be high, and this is often a justification for heavy pesticide use. In these conventional agroecosystems, sole reliance on chemical control for pest management has had numerous adverse consequences, such as the emergence of pesticide resistance among pest and pathogen populations (Davet and Martin 1993 ; Brown et al. 2004 ; Kift et al. 2004), the loss of biodiversity in agroecosystems and adjacent ecosystems (Stoate et al. 2001, 2009), the pollution of water and air, and adverse impacts on human health.

Currently, many governments, including those of the member states of the European Union, are implementing national plans aimed at reducing pesticide applications (Hillocks 2012). Efforts have been made in recent years, especially on cereal crops, to design and assess agricultural systems with reduced dependence on external inputs and compensatory practices (Debaeke et al. 2009). Studies have also been conducted to design and to assess vegetable cropping systems allowing improved management of soilborne pests and pathogens (Tchamitchian et al. 2009 ; Navarrete et al. 2010). However, to our knowledge, no such studies have been undertaken with lettuce crops. The aim of this review is to examine the feasibility of reducing the reliance on pesticides without lowering the productivity of lettuce cropping systems. Although many advances on alternatives to chemical control have been achieved over several decades, these techniques usually have only partial effects on diseases and pests. These methods must be combined to provide efficient control, as proposed by Collange et al. (2011) for the root-knot nematode (*Meloidogyne* spp.), and are sometimes specific to a single pest species. It appears critical, however, to consider all the different species that are likely to threaten the crop because the elements of the agroecosystem interact. Moreover, strategies for disease and pest management must integrate techniques compatible with the biological functions of agroecosystems (Lewis et al. 1997). As a result, knowledge of the mechanisms involved is required to understand the effects of each technique on ecosystem services and particularly on the biological control of pest populations. In addition, these practices and combinations of practices should also meet social and economic expectations to be accepted both by growers and consumers (Pannell 1999). In this review, we first identify themain pests and pathogens that could threaten lettuce crops and the conditions that are conducive to their development. Secondly, we review the techniques, including the use

of pesticides, that are available for the management of these pests and pathogens. The theory supporting these techniques and their action spectra, efficacies, and effects on the agroecosystem properties are presented. Finally, we discuss the best ways to jointly implement these crop protection techniques within lettuce pest and pathogen management strategies, while promoting natural pest and pathogen regulation and other agroecosystem services. We focus on lettuce production under shelter and in open field in the Mediterranean region. However, much of the information can be extrapolated to other temperate climate areas.

2.1 Overview of the lettuce pests and pathogens

Lettuce is prone to many pests and pathogens. Extensive information is available (Blancard et al. 2003 ; Chamont et al. 2010). The aim of this section is not to provide an exhaustive list of diseases and pests but to present those that can cause significant and/or economic losses that lead to many pesticides applications.

2.1.1 Lettuce pathogens

Fungal diseases

One of the most important diseases of lettuce is downy mildew caused by *Bremia lactucae* (Regel). The pathogen may attack the plant throughout its crop cycle. The primary inoculum typically consists of airborne sporangia from diseased plants of the genus *Lactuca* located close to the crop or of mycelia present on plant debris in the soil. The sporangia of *B. lactucae* are typically released from the underside of leaves by sporangiophores, which form a white felt-like layer (Figure 2.1) (Blancard et al. 2003). The optimal conditions for sporulation are a high relative humidity and a temperature from 5 to 15 °C depending on the isolate (Nordskog et al. 2007). Propagules transported by wind ensure secondary contaminations within the crop (Blancard et al. 2003 ; Crute 1992a).

FIGURE 2.1: *Bremia lactucae* sporangiophores forming a white, felt-like layer on the underside of a lettuce leave.

Sclerotinia sclerotiorum (de Bary), *Sclerotinia minor* (Jagger), *Botrytis cinerea* (Pers.), *Rhizoctonia solani* (Kühn), and *Pythium tracheiphilum* (Matta) are important soil-borne fungal pathogens (Blancard et al. 2003). *S. minor* and *S. sclerotiorum* cause lettuce drop and are of major concern for the cultivation of lettuce because they may affect a wide range of plant species

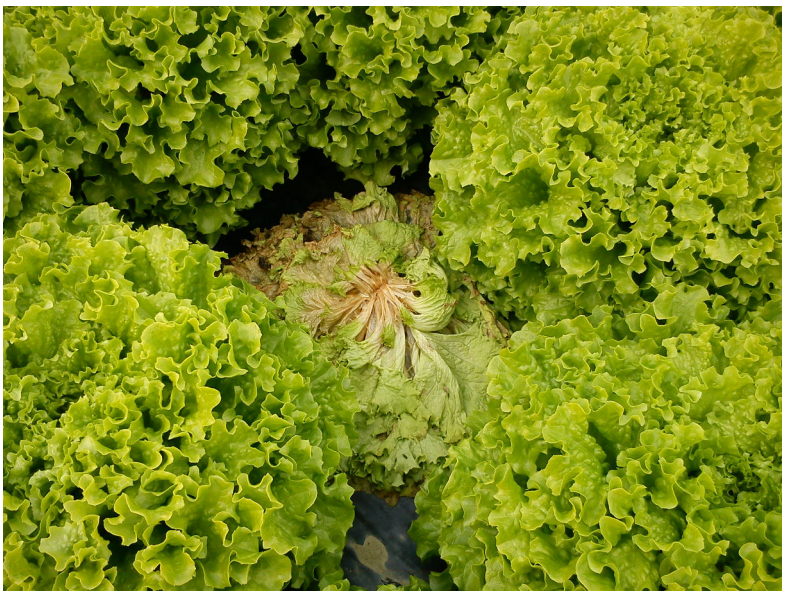

FIGURE 2.2: Extended basal rot caused by *Rhizoctonia solani*, leading to the complete drop of lettuce.

and their sclerotia may remain latent into the soil for more than 8 years (Melzer et al. 1997 ; Bolton et al. 2006). Furthermore, the five pathogens cited above are involved in the development of a shared symptom of basal rot (Figure 2.2). The populations of these pathogens fluctuate depending on the growing season. The sclerotia and mycelia of *R. solani* are most frequently found in soil in the summer, whereas the sclerotia and mycelia of *B. cinerea* are most abundant in the winter, when *B. cinerea* injury to the lettuce leaves is enhanced by cool and moist conditions (Van Beneden et al. 2009). Additionally, *Fusarium oxysporum f. sp. lactucae* is a host-specific lettuce pathogen that has been emerging in Europe, notably in Italy, since 2002 (Garibaldi et al. 2002). This pathogen, a causal agent of Fusarium wilt, is promoted by high temperatures and can cause leaf chlorosis and eventually plant death. It can be transmitted by seeds (Garibaldi et al. 2004) or by resting forms, which can be propagated by tillage tools (Scott et al. 2012). Similarly, *Verticillium dahliae* (Kleb), the causal agent of verticillium wilt, can survive for 10 years in the soil as microsclerotia and be propagated. Although *F. oxysporum f. sp. lactucae* and *V. dahliae* are currently geographically localized, they could become widespread problems in the coming years. *Golovinomyces cichoracearum* (DC), causal agent of powdery mildew, is perceived as a minor pathogen butmay also cause damage on lettuce. The proper temperature range for its development is 18–25 ˚C, so it occurs mainly in the summer and early autumn (Lebeda and Mieslerova 2011). Finally, the fungus *Olpidium brassicae* (Woronin) is not a direct threat to lettuce, but it is a vector for two lettuce viruses that can cause significant damage : Mirafiori lettuce virus, responsible for “big vein” disease ; and lettuce big-vein associated virus, suspected to be the agent of ring necrosis (Lot et al. 2002 ; Verbeek et al. 2012). This fungus is able to remain dormant in the soil or on plant debris for several years in the form of chlamydospores (Blancard et al. 2003).

Bacterial diseases

Lettuce crops are also prone to bacterial diseases. *Pseudomonas cichorii* (Swingle) is the causal agent of varnish spot and *Xanthomonas campestris pv. vitians* (Brown) is the causal agent of bacterial leaf spot of lettuce. Both develop under wet conditions and temperature ranging from 20 to 25 ˚C and from 26 to 28 ˚C, respectively, for *P. cichorii* and *X. campestris pv. vitians.* Seeds, crop residues, or weeds can be sources of inoculum (Blancard et al. 2003 ; Toussaint et al. 2012 ; Barak et al. 2001).

2.1.2 Lettuce pests

Many pests, whether host-specific or not, can also be potential threats depending on the cropping period. These pests include several aphid species, such as *Nasonovia ribisnigri* (Mosley), specific to lettuce ; *Myzus persicae* (Sulzer), the green peach aphid ; *Aulacorthum solani* (Kaltenbach) ; *Macrosiphum euphorbiae* (Thomas) ; and *Hyperomyzus lactucae* (L.). Temperature is the factor that affects most the development of aphids. The highest growth rate of *N. ribisnigri* populations occurs between 20 and 24 ° C (Diaz and Fereres 2005). In addition to feeding damage and the loss of product quality due to their presence when the lettuce is marketed, aphids are also vectors of viruses, such as the lettuce mosaic virus. Moreover, *Pemphigus bursarius* (L.) is an aphid that attacks lettuce roots. The presence of poplars, its main host, close to the crop fosters its occurrence on lettuce, especially at the end of spring. The larvae of several moths [*Autographa gamma* (L.), *Helicoverpa armigera* (Hübner), and *Mamestra brassicae* (L.)] and slugs (*Deroceras* sp. and *Arion* sp.) also cause feeding damage to lettuce. In a production area such as southeastern France, these pests are present mainly in the spring and fall, with a greater occurrence in open fields. In addition, the thrips *Frankliniella occidentalis* (Pergande), the leafminers *Liriomyza* spp., and the whiteflies *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci*(Gennadius) can also occasionally cause significant damage (Palumbo et al. 1997 ; Mou and Liu 2004 ; Costa et al. 1993). *F. occidentalis* is also a vector of tomato spotted wilt virus, which can cause significant yield losses, especially in the summer. Finally, plant parasitic nematodes (in particular in the genus *Meloidogyne* spp.) can attack lettuce crops (Koenning et al. 1999 ; Djian-Caporalino 2012). The development of the nematodes is enhanced by crop used in rotation in southeast of France ; indeed, Solanaceae (tomato or eggplant or pepper) and Cucurbitaceae (melon or cucumber) are susceptible to the same root-knot nematodes. The composition of lettuce pests and pathogens that could threaten the crop at some point changes throughout the year. Across the entire Mediterranean region, diseases such as downy mildew and grey mold tend to be more problematic in the winter, whereas pest pressure is more significant in the spring and fall. It appears necessary to clearly identify the climatic conditions suitable for pest and disease development to know which period is favorable for pests and pathogens (Table 2.1).

However, in addition to climatic conditions, other factors such as crop history, landscape framework, and soil type also affect the composition of lettuce enemies. The identification of pest and pathogen is the first step to design innovative lettuce cropping systems less dependent on pesticides.

2.2 Techniques for the management of lettuce pests and diseases

2.2.1 Limiting the invasion of pests and pathogens

Installing physical barriers

Tillage and crop residue management The simplest and oldest method to reduce primary inoculum is to remove and destroy crop residue (i.e., unharvested leaves and pivot roots) after harvest. This sanitation method can significantly reduce the incidence of diseases ; however, the method is seldom used because it is time consuming. Some cultural practices that affect the structure of the environment can also impact the primary inoculum of lettuce pathogens. For example, tillage that buries crop residues may decrease the survival of stress-resisting forms of the pathogens (Adams 1987 ; Imolehin and Grogan 1980). However, deep and repeated plowing also induces an increased incidence of some diseases. Indeed, plowing may bring viable sclerotia to the surface and also disperse sclerotial aggregates, thus increasing the probability that the sclerotia come close enough to a host plant to infect it (Subbarao et al. 1996 ; Wu and Subbarao 2003). In addition, intensive tillage may also have a negative impact on beneficial soil organisms. Chan (2001) has reviewed the effects of tillage on earthworm populations and reported that repeated deep plowing can reduce their abundance and diversity, while Rodriguez et al. (2006) noted a deleterious effect of such plowing on the abundance of arthropods, especially on spiders and

Main pests and pathogens of Lettuce	Climatic conditions fostering pest or pathogen development	Risk of occurrence in the Mediterranean area					
		Open field			Under shelter		
		Spring	Summer	Early automn	Late automn	Winter	Early spring
Bremia lactucae	Low temperature and high humidity Optimal temperatures: 5-15°C (Nordskog et al. 2007)			+++	++	+++	+
Sclerotinia sclerotiorum and *S. minor*	High soil moisture For *S. sclerotiorum*: development temperature ranges from 10 to 25°C. Optimal temperature: 15°C (Bolton et al. 2006) For *S. minor*: development temperature ranges from 6 to 30°C. Optimal temperature: 18°C (Hao et al. 2003)			++	+	+	++
Botrytis cinerea	High relative humidity and low temperature Optimal temperature: 10-20°C (Elad and Shtienberg 1995)				+	+++	+
Rhizoctonia solani	Optimal temperature: 20-30°C (Grosch et al. 2004)			+	++	+	++
Pythium tracheiphilum	High soil moisture Development temperature ranges from 5 to 43°C. Optimal temperature: 20-24°C (Blancard et al. 2003)			+	++	+	+
Fusarium oxysporum	Optimal temperature: 20-30°C (Scott et al. 2010)		+				
Golovomyces cichoracearum	Optimal relative humidity: 95-98% Optimal temperature: 18-25°C (Lebeda and Mieslerova 2011)			++			
Viruses transmitted by *Olpidium brassicae*	Low temperature and high humidity. Optimal temperature: 10 - 16°C (Chamont et al. 2010)					+++	
Aphids – *Nasonovia ribisnigri* and *Myzus persicae*	For *N. ribisnigri*: development temperature ranges from 3 to 35°C. Optimal temperature: 26°C (Diaz et al. 2007) For *M. persicae*: development temperature ranges from 6 to 37°C. Optimal temperature: 27°C (Davis et al. 2006)	+++	+	++	+	+	+++
Meloidogyne spp.	Development temperature ranges from 5 to 38°C. Optimal temperature: 18 -27°C (Blancard et al. 2003)	+	+	++	+		+
Helicoverpa armigera	For *H. armigera*: Optimal temperature: 27,5°C (Mironidis and Savopoulou-Soultani 2008)	++	+	+++			

TABLE 2.1: Climatic conditions fostering pest and pathogen development and epidemic risks in Mediterranean lettuce crops depending on production type and season. This table allows the identification of pests and pathogens that could threaten the lettuce crop at some point of the year and for a specific production framework. However, other factors, such as crop history, landscape characteristics and soil type, also affect the composition of lettuce pests and pathogens. Potential risk (+) ; significant risk (++) ; major risk (+++).

hymenopteran parasitoids, under the Mediterranean climate. Although the effects of intensive tillage are well documented, the short-term consequences of reduced tillage on lettuce pests and yield are rarely addressed in the literature. Further studies are needed to determine the most favorable tillage protocols for balancing the trade-offs among pest control, yield, and other ecosystem services, such as nutrient cycling and soil restructuring by arthropods and earthworms.

Insect-proof nets Insect-proof nets may provide an effective protection against pests both in protected crops and in open fields (Weintraub and Berlinger 2004). This technique aims to prevent infestation by establishing a physical barrier between the invading pest and the crop. This barrier also protects the plants from contamination by pest-transmitted viruses, such as those carried by aphids. Nevertheless, nets present the drawback of modifying the microclimate in the canopy (increased temperature and relative humidity) by limiting air flow.

Colored mulch material The behavior of some pests, such as winged aphids, is partially conditioned by visual stimuli. According to Döring et al. (2004), two visual impediments may affect host-plant infestation by aphids. First, the lower the contrast between the target and its background, the more difficulty aphids have to land on their target. Second, a background with high reflectance of short wavelength light disturbs host recognition. For example, when a trap used to simulate a green host plant was surrounded by a white or silver background, significantly fewer *M. persicae* were caught than when the background was black or red or consisted of bare soil. In addition, Fricke and Piepenbrock (2005) observed a fivefold reduction in populations of winged aphids (species not specified) on lettuce when a silver mulch is used instead of a green one. Furthermore, Döring et al. (2004) argued that the structure of the background may also act as a barrier to infestation because aphids that land on a smooth structure like a leaf tend to probe and, if unsuccessful, quickly depart, whereas aphids that land on a rough structure, such as soil, move around the area before probing. In addition to limiting the growth of weeds and reducing evaporation, mulch can play a significant role in the control of pest populations. For this purpose, mulch color, structure, and contrast with the crop must all be considered. Fricke and Piepenbrock (2005) reported a trend for increased caterpillar populations (including *M. brassicae* and *A. gamma*) in broccoli fields with ultraviolet-reflecting mulches. Studies should be conducted to determine which mulch material best prevents pest infestations and to evaluate how mulching affects the plants, the pests, and the natural enemies of those pests.

Ultraviolet protection cover Light, particularly in the ultraviolet range with wavelengths from 280 to 400 nm, can affect organisms, trophic interactions, and thus agroecosystem functions (Paul and Gwynn-Jones 2003 ; Lagier 2005). It may play an important role in establishing relationships between plants and pathogens or pests (Raviv and Antignus 2004). Changes in the spectral characteristics of incident light, obtained for example by filtering out ultraviolet wavelengths, can alter several behavioral traits of pest insects, such as orientation and host or food finding (Díaz and Fereres 2007a). Antignus et al. (1996) have shown that the absence of ultraviolet light can disrupt the navigation of some insects, such as thrips and whiteflies. Diaz et al. (2006) have demonstrated that the use of ultraviolet-absorbing films in lettuce fields can reduce the abundance of aphids (*M. euphorbiae*), thrips (*F. occidentalis*), and *A. gamma* larvae. Similarly, the modification of electromagnetic radiation within greenhouses may impact pathogen populations, although their responses are not necessarily consistent (Raviv and Antignus 2004). Nevertheless, ultraviolet- B generally tends to decrease the survival of spores of pathogenic fungi, whereas ultraviolet-A appears to enhance pathogen reproduction (Paul and Gwynn-Jones 2003). Regarding lettuce pathogens, ultraviolet-B (280–320 nm) decreases the viability of *B. lactucae* sporangia (Wu et al. 2000) and *S. sclerotiorum* ascospores (Caesar and Pearson 1983), while ultraviolet-A may increase the production of spores by *B. cinerea* (Nicot et al. 1996 ; Paul and Gwynn-Jones 2003). Changing the light spectrum, particularly in the ultraviolet-B range, also changes the defense-related metabolism of plants (Paul and Gwynn-Jones 2003) ; for example, an increase in ultraviolet-B may increase the production of phenolic acids and flavonoids known to be involved in defenses against herbivores and pathogens. In addition, plants grown under ultraviolet-opaque films may have a modified morphology or taste (Paul et al. 2005), which

could discourage the adoption of this technology by lettuce growers. The establishment of a zero-ultraviolet environment under shelter is possible because spectral conditions can be easily modified using screens that filter ultraviolet radiation. In fact, most currently used plastic films partially absorb ultraviolet radiation (Tsormpatsidis et al. 2010), although the effect of ultraviolet blocking materials on the various lettuce pathogens, pests, and beneficial organisms still remains to be investigated (Díaz and Fereres 2007a). These issues must be addressed before considering the integration of this technique into pest and disease management strategies.

Eliminating persistent forms of pests and pathogens before lettuce planting

Rotation Plant species diversity within agroecosystems plays a major role in the management of crop pests and diseases (Ratnadass et al. 2012 ; Letourneau et al. 2011). Increasing diversity through crop rotation is one of the ways to reduce the detrimental effects of pests and diseases. Koike et al. (1997) investigated the potential of barley, fodder radish, and fava beans as winter crops to control *S. minor* and found that the planting of these crops, which are not *S. minor* hosts, helps to maintain a low incidence of disease in the next lettuce crop. Similarly, in a study conducted in California, Hao and Subbarao (2006) have shown that growing broccoli before lettuce could reduce not only the number of *S. minor* sclerotia in the soil but also the incidence of disease on the following lettuce crop. All these crops may be appropriate candidates for the diversification of rotations ; however, their susceptibility to other lettuce pests should be considered (Koike et al. 1997).

Solarization Solarization is a passive physical technique used to reduce soil-borne populations of pathogens (or pests) before a crop cycle. The principle is to increase soil temperature to lethal levels by trapping solar radiation with a polyethylene film (Figure 2.3).

FIGURE 2.3: Solarization performed under shelter to reduce soil inoculum

The film is laid on wet soil in open fields or under shelters and left on the soil surface for several weeks when the solar irradiance is maximal. Solarization induces changes in the physical, chemical, and biological properties of the soil (Stapleton 2000). The viability of *S. sclerotiorum*,

S. minor, *R. solani* sclerotia and *O. brassicae* (the fungal vector of lettuce big-vein associated virus), root-knot nematode, and many weeds is strongly affected by the high temperature, high soil moisture, and low oxygen levels produced by solarization (Candido et al. 2008 ; Patrício et al. 2006 ; Pares and Bressoud 2010). Nevertheless, a drawback of solarization is that the abundance of some beneficial organisms (e.g., arthropods) may also be decreased (Seman-Varner 2006). However, solarization-induced changes in the soil biota also promote heat-resistant or tolerant species and fast recolonizers, such as certain bacteria (*Bacillus* spp.), fungi (*Trichoderma* spp.), and free-living nematodes, that can enhance pest control services (Stapleton 2000). Solarization is well suited to the Mediterranean climatic conditions (Candido et al. 2008). It also fosters nutrient cycling because prolonged high temperatures promote nitrogen mineralization in the soil, which in turn can benefit the succeeding crop and even increase its yield (Hasing et al. 2004 ; Patrício et al. 2006).

Biofumigation Biofumigation is typically carried out by growing an intermediate crop, which is shredded and incorporated into the soil. Once in the soil, the plant residues degrade, releasing natural compounds which can kill plant pathogens or pests. For this purpose, the potential of crucifers (particularly species in the genus *Brassica*) has been extensively studied. They have proven useful against several of the main soil-borne pathogens of lettuce, such as *Sclerotinia* spp. and *R. solani* (Sarwar et al. 1998) and against root-knot nematodes (Zasada et al. 2010). Although certain species of *Brassica* may be hosts of root-knot nematodes and *Sclerotinia* spp., the incorporation of their crop residues promotes the release of isothiocyanates, which have nematicidal and fungicidal effects, and can also show insecticidal, phytotoxic, and antibiotic properties. Several isothiocyanates can inhibit the growth of *S. sclerotiorum* mycelia and reduce sclerotial viability and germination. In field studies, seven isothiocyanates were shown to decrease the incidence of lettuce drop by 63 % to 83 % (Kurt et al. 2011). Biofumigation is effective only if the Brassica crop has high biomass and glucosinolate content. Optimal biofumigation also requires fast hydrolysis of the glucosinolates, which can be achieved with finely ground plant tissues, high temperature, and high soil moisture (Gimsing and Kirkegaard 2009). Thus, although farmers have begun to adopt the use of *Brassica* (especially *Brassica juncea*) residues as biofumigants, the effectiveness of this method is still variable. Other plants in addition to *Brassica* species can be used as biofumigants, including those that contain cyanoglucosides that release hydrogen cyanide upon degradation, as cyanide has broad biocidal effects. Viaene and Abawi (1998) and Widmer and Abawi (2002) have shown that sudangrass (*Sorghum sudanense* cv. Trudan 8) used as a green manure before a lettuce crop slows the development of root-knot nematode eggs (*Meloidogyne hapla*) and reduces the number of galls on lettuce roots. However, these authors stressed that the decomposition of sudangrass could also have a phytotoxic effect on lettuce and thus eventually reduce yields.

Chemical fumigation Another method to eliminate soil-borne pathogens is chemical fumigation of the soil. Until its ban in 2010 in European Union, methyl bromide was the most-used chemical fumigant for the control of soil-borne fungi such as *R. solani* and *Sclerotinia spp.*, plant parasitic nematodes, and *O. brassicae* (Duniway 2002). Currently, several chemical alternatives to methyl bromide are available ; however, their efficacy may be lower and their spectrum of activity more limited. For example, 1,3-dichloropropene is highly effective against nematodes but exhibits variable efficacy against fungal pathogens (Qiao et al. 2010 ; Zasada et al. 2010). Furthermore, the protective effect of alternative fumigants may not carry over beyond the first crop. This was observed for example in a lettuce field experiment (Ceustermans et al. 2010). For these reasons, combinations of several soil disinfectants are commonly used.

2.2.2 Limitation of pest and pathogen development on plants

As mentioned earlier, to eliminate the sources of primary inoculum within and close to the crop, it is critical for the grower to purchase or produce healthy lettuce seedlings. Currently, the protection of seedlings in nurseries is primarily achieved by pesticide applications. After securing

the sanitary quality of seedlings, several techniques can be used during the culture to limit pest and pathogen developments.

Limitation mediated by the plant

Genetic resistance Genetic resistance through breeding programs is commonly exploited for pest and disease management because it is easy for growers to use and has little to no negative effect on the environment (Cuartero et al. 1999). For lettuce, resistance to pests and diseases is one of the major objectives of the breeding programs. Accordingly, breeding programs for resistance to downy mildew started in 1950 in England (Crute 1992b) and still continue worldwide. The first sources of resistance were found in old lettuce varieties (*L. sativa*), followed by *Lactuca serriola*, a related and compatible wild species, which has been extensively used (Crute 1992b). More recently, resistance genes from *Lactuca saligna*(Jeuken and Lindhout 2002) and *Lactuca virosa* (Maisonneuve 2003) have been studied, but their introgression in lettuce is more difficult (Maisonneuve 1987 ; Maisonneuve et al. 1995). The list of genetic resistance to disease in these species has been recently reviewed by Lebeda et al. (2009). Currently, breeding efforts focus primarily on resistance to downy mildew, with the identification and pyramiding of the major resistance genes involved in a specific gene-for-gene interaction. These programs are leaded by private companies. However, resistance breakdown caused by new virulent strains of *B. lactucae* is common and leads to a rapid turnover of lettuce varieties (Michelmore and Wong 2008).

Resistance to the lettuce mosaic virus has also been introduced in European field cultivars from a Latin lettuce, Gallega de Invierno, and in American crisphead cultivars from the Egyptian wild lettuce *L. sativa* (Dinant and Lot 1992). Resistance to the leaf aphid, *N. ribisnigri*, due to the Nr gene identified in *L. virosa* (Eenink et al. 1982), has been introduced in many European varieties for summer field culture (Arend and Schijndel 1999). This resistance has recently been circumvented by a new biotype named Nr : 1, but searches to find Nr : 1 resistance sources are progressing (Cid et al. 2012). Finally, in a few varieties, other resistance genes have been introduced to protect the plant against *P. bursarius* (Ellis et al. 1994) or against *Fusarium oxysporum f.* sp. *lactucae* (Scott et al. 2012) in exposed areas.

Many other examples of genetic resistance have been identified and studied in *Lactuca* spp. (Pink and Keane 1993) but have not yet been used in commercial varieties. This is the case for genetic resistance to *Sclerotinia* spp. Complete resistance has not been observed but some varieties of *L. sativa* exhibit different susceptibilities to the disease, suggesting the presence of partial resistance genes. The lowest susceptibilities to the disease may be partially correlated with morphological features of the plant, such as early bolting or upright plant architecture, which confer an ability to avoid the pathogen. Nevertheless, the integration of these morphological characteristics into breeding programs is not desirable because these traits do not meet the needs of producers and consumers (Grube and Ryder 2004). Differences in susceptibility to *Sclerotinia* spp. have also been observed in lettuce varieties that display similar architectures (Grube and Aburomia 2004 ; Hayes et al. 2010). These observations support the involvement in plant defenses of partial resistance genes, which do not influence plant architecture and could be more easily integrated into breeding programs ; however, to our knowledge, none of these genes have yet been identified. According to Whipps et al. (2002), a way to distinguish these two types of resistance expression is to perform screenings using two separate methods (by natural contamination vs. by inoculations). Natural contaminations can highlight partial resistance based on plant architecture, whereas inoculations can reveal partial resistance that arises from defenses within the plant tissues.

As mentioned above, pests and pathogens could overcome genetic resistance, especially those which involve one or a few genes. To enhance the sustainability of genetic resistance, several strategies were proposed : the identification and regular introduction of new resistance genes, the combination of a maximum number of resistance genes in one genotype, and the introduction of partial resistance (Dogimont et al. 2010 ; Pink 2002). These partial resistance genes could be more sustainable because they are less specific and exert less selection pressure on pest populations. In this regard, Hand et al. (2003) have detected several quantitative trait loci (QTL) of resistance to both *B. lactucae* and *M. persicae* in a population from a cross between two varieties of *L.*

sativa. Then, by crossing *L. sativa* (susceptible) and *L. saligna* (non-host resistance), Zhang et al. (2009) were able to combine backcross inbred lines containing three QTL from *L. saligna* and demonstrate a complete resistance to the two strains of *B. lactucae* tested (Bl :14 and Bl :16). These authors have shown the potential of vertical resistance for the sustainable control of lettuce diseases and pests ; however, the non-host nature of *L. saligna* resistance to *B. lactucae* has been challenged by Petrezlova et al. (2007). Another way to sustain plant defenses is to combine several varieties with complementary resistances in the field to dilute pathogen pressures (Schaerer 2008 ; Maisonneuve et al. 2006). However, few studies have been conducted on lettuce crops, and the genetic resources of resistance are still poorly characterized, except for *B. lactucae.*

Resistance inducers The expression of plant resistance against diseases and pests may be constitutive or inducible. Constitutive plant defenses are provided by both inherent physical barriers and antimicrobial metabolites. By contrast, induced resistance refers to a plant defense that must be initiated by a stimulus, which can be a direct interaction with a pest or the perception of a signal from chemical compounds or biological organisms. Induced resistance can be divided into two categories (Hammerschmidt 1999 ; Walters and Heil 2007) : (1) systemic acquired resistance is generally effective against a broad spectrum of pathogens, requires a salicylicacid signal, and involves the production of pathogenesisrelated proteins, such as chitinases and glucanases ; and (2) induced systemic resistance is initiated by interactions between plant roots and beneficial soil-born organisms, such as plant-growth-promoting rhizobacteria, and has a signal that is generally mediated by a jasmonate/ethylene pathway. Indeed, salicylic acid, jasmonic acid, and ethylene are the main phytohormones involved in the signaling pathways of the induced defenses, and crosstalk between these pathways is expected to allow plants to modify their defense responses (Pieterse et al. 2009). The application on crops of compounds or organisms (named resistance inducers below) able to activate the inducible signaling pathways could strengthen plant defenses (Vallad and Goodman 2004 ; Walters et al. 2005, 2013). In the case of lettuce, several compounds, such as β -amino butyric acid or potassium phosphonate (K_2HPO_3), were identified as resistance inducers against *B. lactucae*. Both compounds cause a dose-dependent systemic resistance, which is completely efficacious in laboratory experiments up to 15 days following inoculation (Pajot et al. 2001). Under field conditions, β -amino butyric acid was also shown to effectively reduce *B. lactucae* infections on lettuce in a dose-dependent manner (Cohen et al. 2007). β-amino butyric acid, as a resistance inducer against *B. lactucae*, appears to act through a systemic acquired resistance pathway because the defense reaction is both local and systemic, not strain specific, and involves pathogenesis-related proteins. This defense inducer leads to a massive accumulation of callose between the mesophilic cells if mycelium is detected (Cohen et al. 2010, 2011). While the modes of action and resistance mechanisms of β -amino butyric acid have been clearly identified, those of potassium phosphonate are still unsolved. The phosphonate seems to act both through a direct fungitoxic effect by the release of phosphonate ions (HPO3-) known for their antimicrobial activities (inhibition of germination) and through the induction of systemic acquired resistance (Reuveni and Reuveni 1998). Other phosphate salts were found to be inducers of systemic acquired resistance in cucumbers, causing hypersensitivity reactions on the treated foliage and increased levels of free salicylic acid in the leaf tissues (Gottstein and Kuc 1989 ; Orober et al. 2002). Many other studies reviewed by Deliopoulos et al. (2010) have shown the effect of phosphate salts on other plant defenses pathways against several fungal pathogens. Fungi or bacteria can also induce resistance. An extensive description of the mechanisms underlying induced systemic resistance is beyond the scope of this review, and readers are invited to refer to an excellent recent review (Shoresh et al. 2010 and references therein).

Bacillus subtilis and other *Bacillus* spp. are stimulators of induced systemic resistance, which enhances resistance against various pests. The production by *B. subtilis* of lipopeptides, including surfactin and fengycin, is at least partially responsible for inducing plant resistance against *B. cinerea* (Ongena et al. 2007). Similarly, *T. harzianum* T39 triggered an induced systemic resistance against B. cinerea in lettuce, pepper, tobacco, beans, and tomatoes by delaying or completely stopping the development of lesions (De Meyer et al. 1998). Furthermore, Yogev et al. (2010) demonstrated the induction of plant resistance against *Fusarium oxysporum* and *Botrytis cinerea* in melon by some composts. The mechanisms of resistance induction by composts

remain unclear. Induced resistance is expected to be difficult to overcome by pest and pathogen populations. Walters and Heil (2007) suggested that the selection pressures exerted on pest populations are minor because involved defense mechanisms are controlled by several genes and so appear difficult to overcome. In addition, the heterogeneity of responses to the induction of defenses allows the preservation of « refuges » (non-induced plants), limiting induced resistance breakdown. Recent laboratory experiments have also shown that different lettuce varieties vary in the intensity of their response to the same defense inducer (Maisonneuve et al. 2013). Finally, although induced resistance appears to have lower fitness costs than constitutively expressed resistance, several authors have demonstrated that induced systemic resistance and systemic acquired resistance also have costs for plants. Such costs may be linked to the production and transport of defense compounds or are ecological costs incurred when the induction of defenses disturbs the interaction between plants and their beneficial organisms (Heil and Baldwin 2002 ; Walters and Heil 2007). To avoid increasing fitness costs that could result in yield losses, the induction of defenses in the field by chemical compounds or biological organisms should be utilized according to incurred epidemic risks.

Fertilization Fertilization affects plant–pathogen as well as plant–pest interactions, but the mechanisms underlying the effects of fertilization on the susceptibility of plants to diseases and pests are still poorly understood. Fertilization is a determinant of the plant defense capabilities, which affect plant growth, resistance mechanisms, and pest population dynamics (Dordas 2008 ; Altieri and Nicholls 2003 ; Walters and Bingham 2007). For pests, Pakarinen et al. (1990) have shown that the lettuce leaves most palatable for slugs (*Deroceras* sp.) were those that had the highest contents of nitrogen and phosphorus. There are currently no results on aphid behavior on lettuce ; however, different levels of soluble nitrogen in the leaves of apple and peach have affected the reproduction rates of aphids (Rutz et al. 1990 ; Sauge et al. 2010). Sauge et al. (2010) have shown that the optimal nitrogen fertilization for *Myzus persicae* populations on peach had an intermediate value (6 mM of nitrate in the fertigation solution) and that low or high values diminished aphid population density. Regarding the effect of nitrogen fertilization on fungi and oomycetes, available studies report apparently inconsistent results which vary depending on the crop species, pathogen species and strain aggressiveness (Lecompte et al. 2010), the plant stages (Dordas 2008), and the input forms (Huber and Watson 1974). On lettuce specifically, Lecompte et al. (2013) have shown that damage on leaves inoculated with *B. cinerea* or *S. sclerotiorum* increased with the level of nitrogen fertilization applied. They suggested that, at least for *S. sclerotiorum*, the host susceptibility was linked to its sugar content, which varied depending on the nitrogen fertilization. Actually, the balance among the different allocations of nitrogen in the plant could be responsible for the variable responses found among the above studies. Indeed, Walters and Bingham (2007) suggested that high plant nitrogen content promotes the development of pathogens by providing them more nutritional resources and that it can also affect the production rates of compounds involved in plant defenses. Despite some inconsistent reports, numerous authors agree that cultural practices related to plant nutrition (e.g., fertilization and irrigation) may significantly affect crop susceptibility and could be used to manage diseases and pests (Walters and Bingham 2007 ; Sauge et al. 2010). However, currently available data for lettuce are not sufficient and the opportunities of optimizing field fertilization to reduce pest damage remain to be investigated.

Limitation mediated by the abiotic environment

The germination of infectious forms (spores or sclerotia) of numerous pathogenic fungi depends on climatic factors such as humidity and temperature. Indeed, one of the most important factors for the germination of *B. lactucae* sporangia is the duration of leaf wetness (Scherm and Bruggen 1994). Three hours of leaf wetness are necessary for the sporangia to germinate and penetrate the host plant (Wu et al. 2002). When the air flow under a shelter is restricted, the duration of leaf wetness tends to increase and to promote the incidence of the disease (Boulard et al. 2004). The duration of leaf wetness is also an important factor for *B. cinerea* spore germination (Elad and Shtienberg 1995). Therefore, the ventilation of greenhouses is a significant tool for

the management of these diseases. Tools to forecast the epidemic risks of *B. lactucae* infection have been developed and are based mainly on climatic conditions (e.g., temperature, relative humidity, leaf wetness duration, and solar radiation) (Kushalappa 2001 ; Wu et al. 2002). These models predict the optimal timing of phytosanitary treatments and could reduce their numbers compared with a calendar-based treatment strategy (Hovius et al. 2007) ; however, the models are not now commonly used.

Irrigation is a cultural practice that also affects the microclimate (Scherm and Bruggen 1995). Irrigation applied directly at the soil level (furrow or drip irrigations) instead of above the canopy (sprinkler irrigation) reduces leaf wetness and thereby moderates the incidence of downy mildew caused by *B. lactucae*. In addition, Scherm and Bruggen (1995) revealed that climatic conditions created by a drip irrigation system are less favorable to the development of *B. lactucae* than are those established following furrow irrigation. Accordingly, studies conducted by Wu and Subbarao (2003, 2006) showed that drip irrigation (subsurface and surface drip) can reduce by 50 % the incidence of lettuce drop caused by *Sclerotinia* spp. compared with conventional irrigation (sprinkler or furrow). The reduced moisture at the soil surface and the increased soil temperature under subsurface drip irrigation could reduce the germination of sclerotia (Wu and Subbarao 2003).

As already demonstrated for *S. minor* on peanut crops (Dow et al. 1988 ; Maas et al. 2006), plant spacing and particular architectural features of the crop (e.g., upright lettuce vs. plants with flat bases) could influence the impact of soil-borne diseases by modifying the microclimate under the canopy. Eventually, the management of the macroclimate (under shelter) or the microclimate (under the canopy) may become an important method to prevent disease development.

Removal of pests and pathogens

Biological control Biological control is based on biotic interactions between pests or pathogens and their antagonists. Biological control agents may be predators, parasites, or competitors (Table 2.2).

Several biological control techniques could be used to manage populations of lettuce pests and pathogens. The endemic populations of biological control agents could be promoted by habitat manipulation. The biological control agents could also be introduced preventively or curatively.

Conservation biological control The principle is to provide suitable resources and habitats for the timely establishment and development of endemic biological control agent populations (Landis et al. 2000). Plant diversity can play a major role in attaining these goals (Ratnadass et al. 2012). Habitat manipulation tactics have to consider the foraging behavior of the natural enemies. Laboratory and field studies have shown that sugar and pollen improve the fecundity of aphid parasitoids and that aphid parasitism decreases when the distance to resources increases (Tylianakis et al. 2004). Thus, pollen- or nectar-rich flowering plants are often used. The morphology of flowers is also a criterion for the selection of plants because the natural enemies of pests require easy resource access (Landis et al. 2000). In addition to resource provision for the enemies of pests, the introduction of different plant species in the vicinity of a field can also serve as refuges for those natural enemies to take shelter during adverse conditions (e.g., high or low temperature or pesticide applications). However, this plant diversity can sometimes be detrimental by supporting pest populations (Landis et al. 2000) ; it should accommodate the needs of the natural enemies of the key pests and avoid attracting other crop pests.

Concerning lettuce crops, the introduction of plants which provide resource subsidies to pest predators and parasitoids are the most studied strategies for habitat manipulation (Sengonca et al. 2002 ; Pascual-Villalobos et al. 2006 ; Masetti et al. 2010). Pascual-Villalobos et al. (2006) found that planting strips of *Corandium sativum* and *Chrysanthemum coronarium* within a lettuce crop tended to promote syrphid populations, but the results were not statistically significant. However, they also observed that predators appeared only after the establishment of aphids. Similarly, Sengonca et al. (2002) showed an increase in populations of adults and predatory larva of four polyphagous predators, including *Chrysoperla carnea*, when weeds (*Artemisia vulgaris*, *Tanacetum vulgare*, or *Urtica dioica*) were planted within and near the lettuce crops. In the same

way, Géneau et al. (2012) demonstrated that two parasitoid wasps of *Mamestra brassicae*, *Microplitis mediator* and *Diadegma fenestrale*, can be enhanced by the presence of nectar-producing species such as *Fagopyrum esculentum*, *Centaurea cyanus*, and *Vicia sativa*.

The authors also showed that these plants do not improve the fitness of *M. brassicae*. Further studies are needed to determine which plant species can be introduced safely to support endemic biological control agents.

Preventive introduction of natural enemies The introduction of banker plants into a crop is a preventive and long-term means of pest biological control. This technique has been studied for aphid control. Banker plants belong to a species which is different from the crop and they are infested with aphid species that do not use the crop as their host but are parasitized by natural enemies that also target those aphids that harm the crop. Thus, parasitoids that develop on the banker plant are present in the field when the crop pests arrive (Frank 2010). As an example, the complex formed by the aphid *Rhopalosiphum padi* (L.), a specific pest of Poaceae, and the host parasitoids *Aphidius colemani* and *Aphidius ervi* can be used against aphid populations that thrive in lettuce crops because *A. colemani* and *A. ervi* are also parasitoids of the main lettuce aphids (*N. ribisnigri*, *M. persicae*, *M. euphorbiae*, and *A. solani*). In this case, the banker plant should be a Poaceae adapted to the climatic conditions of the season ; barley, wheat, and oats are the most commonly used (Frank 2010). The effectiveness of this technique depends on the rate at which the parasitoids are released onto the crop, which depends on the density of the banker plants and on the climatic conditions that influence the development of the parasitoid (Frank 2010).

Biological control agents can also be introduced into the soil to manage soil-borne pathogens. *Coniothyrium minitans* is currently used in lettuce cropping systems to reduce *Sclerotinia spp.*, *B. cinerea*, and *R. solani* propagules (Chitrampalam et al. 2008). This fungus preferentially parasitizes overwintering structures by synthesizing chitinases, glucanases, and antifungal metabolites (Zeng et al. 2012). *Trichoderma harzianum* is also known to disturb populations of *B. cinerea*, *Pythium spp.*, *R. solani*, and *Sclerotinia* spp. in soil, acting either as a competitor or as a parasite (Ozbay and Newman 2004). However, the effects of biological control agents on non-target organisms need to be further explored ; for example, it has been suggested that *T. harzianum* can parasitize species of the arbuscular mycorrhizal fungus (*Glomus* spp.) (Brimner and Boland 2003). Another way to introduce antagonistic organisms is to amend the soil with compost. The suppressive effect of soil-borne diseases by some composts is attributed to several mechanisms involving microbial communities, including competition, parasitism, and antibiosis (Hadar and Papadopoulou 2012). Although many studies have reported a suppressive effect of these composts on several diseases caused by soil pathogens such as *R. solani*, *Sclerotinia* spp., *Verticillim dahliae*, *Pythium* spp., and *Fusarium* spp., composts are currently poorly used (Pane et al. 2013 ; Bonanomi et al. 2007). This may be due to the varying efficacies of composts which are linked to their biotic and abiotic components.

Curative biological control Lettuce pests can also be controlled with the mass release of beneficial organisms in the field, as a curative technique. Many species are known predators or parasitoids of lettuce aphids (Table 2.2). The larva of the generalist predator *Chrysoperla carnea* can ingest up to 270 *Myzus persicae* aphids per day (Liu and Chen 2001). Aphidophagous syrphids are other generalist predators that could participate in the management of the lettuce aphid. However, because only the larval stages are predators of aphids, additional food sources appropriate for the other life stages are necessary to support population development. The presence of preys at the time of the mass release is critical.

Application type	Biological control agents	Target	Action	Requirements	References
Soil application	*Coniothyrium minitans*	*Sclerotinia sclerotiorum*, *Sclerotinia minor*, *Rhizoctonia solani*, *Botrytis cinerea propagules.*	Sclerotia parasitism, reduction of disease incidence at the following lettuce crop.	Optimum temperature for parasitism 15-25°C. Latent period of 8 weeks between inoculation and lettuce planting. Need for direct contact with the sclerotia.	(Van Beneden et al. 2010; Jones and Whipps 2002; Chitrampalam et al. 2008; Chitrampalam et al. 2010; Yang et al. 2010)
	Trichoderma harzianum T22	*Pythium* spp. *S. sclerotiorum*, *S. minor*, *R. solani*, *B. cinerea propagules.*	Mycoparasitism, induced systemic resistance., competition	Optimum temperature 25-30°C. The lowest temperature for *Trichoderma sp.* activity is 8°C.	(Ozbay and Newman 2004; Paulitz and Belanger 2001; Klein and Eveleigh 1998)
Foliar application	*Trichoderma harzianum T39*	*B. cinerea*	Competition, lytic enzyme production, induced systemic resistance	Optimum temperature 25-30°C. The lowest temperature for *Trichoderma sp.* activity is 8°C.	(De Meyer et al. 1998; Paulitz and Belanger 2001; Klein and Eveleigh 1998)
	Bacillus subtilis	*B. cinerea*, *S. sclerotiorum*, *R. solani.*	Production of antifungal metabolites, antagonism, induced systemic resistance.	No irrigation on foliage because the bacterium is washable.	(Fiddaman et al. 2000; Zhang and Xue 2010)
	Bacillus thuringiensis	*Helicoverpa armigera*	Bioinsecticide (insecticidal crystal protein)	No irrigation on foliage because the bacterium is washable. Toxins must remain on the plant to be ingested by phytophagous organisms.	(Lacey et al. 2001)
	Steinernematidae / Heterorhabditidae	*H. armigera*	Entomopathogenic nematode associated with symbiotic bacteria	Conditions that promote the survival and mobility of nematodes (high humidity, low radiation, low air flow). Applications are more appropriate under tunnel than in open field.	(Arthurs et al. 2004)
Introduction on banker plants or mass release	*Aphidius ervi*	*Myzus persicae*, *Macrosiphum euphorbiae*, *Aulacorthum solani*	parasitoid	The lower temperature threshold for development is 6.6°C (mummy to adult development). Complete development in 73° days.	(Nebreda et al. 2005; Kavallieratos et al. 2004; Sigsgaard 2000)
	Aphidius matricariae	*M. persicae*	parasitoid	The lower development threshold is 3.51°C. The rate of parasitism increases with the temperature up to 25°C.	(Nebreda et al. 2005; Kavallieratos et al. 2004; Zamani et al. 2007)
	Praon volucre	*M. persicae*, *M. euphorbiae*	parasitoid	The lower temperature threshold for development is 5.5°C (mummy adult development). Complete development in 70°days.	(Nebreda et al. 2005; Kavallieratos et al. 2004; Sigsgaard 2000)
	Aphidius colemani	*M. persicae*, *Nasonovia ribisnigri*	parasitoid	The lower development threshold is 2.65°C. The rate of parasitism increases with the temperature up to 25°C.	(Nebreda et al. 2005; Kavallieratos et al. 2004; Zamani et al. 2007)
	Aphidius hieraciorum	*N. ribisnigri*	parasitoid	not specified	(Nebreda et al. 2005; Kavallieratos et al. 2004)
Mass release	*Chrysoperla carnea*	*M. persicae*, *H. armigera*	Polyphagous predator (larva)	The lower development threshold is 10°C.	(Honek and Kocourek 1988; Liu and Chen 2001; King and Coleman 1989; Reddy and Manjunatha 2000)
	Episyrphus balteattus	*N. ribisnigri*	Polyphagous predator	The lower development threshold is 7.1 °C.	(Hart et al. 1997; Honek and Kocourek 1988; Hopper et al. 2011)

*The lower development threshold may vary among aphid hosts, but is most likely close to the value estimated for *M. persicae*

TABLE 2.2: Main biological control agents of lettuce pests.

Pesticide applications Insecticides and fungicides are the main pesticides commonly used on lettuce crops, weeds being managed mostly mechanically (e.g., through mulches). The active substances belong to different chemical families and can have contact or systemic actions. Their use can lead to the presence of pesticide residues on lettuce leaves (Gonzalez-Rodriguez et al. 2008) ; therefore, the shortest interval allowed between application and harvest is defined for each active ingredient. The main fungicides commonly applied to lettuce crops to manage the oomycetes *B. lactucae* or *Pythium* spp. belong to the dithiocarbamate and strobilurin families. The dithiocarbamates (e.g., mancozeb and methiram) have three complementary modes of action : inhibition of glucose oxidation, nucleic acid synthesis, and fatty acid degradation. The strobilurins (e.g., azoxystrobine) affect fungi through the inhibition of mitochondrial respiration. One strobilurin (pyraclostrobin) and chemicals of the dicarboximide, phenylpyrrole, and anilinopyrimidine families are used to manage *Sclerotinia* spp. and *B. cinerea*. The dicarboximides (e.g., iprodione) affect the osmotic regulation of fungal tissues. The anilinopyrimidines (e.g., cyprodinil and pyrimethanil) inhibit the synthesis of amino acids. Fungicide resistance is a widespread phenomenon in lettuce fields. Resistance to organophosphates and phenylamides has also been identified in *B. lactucae* strains in California, in Italy, and in France (Brown et al. 2004 ; Cobelli et al. 1998 ; Leroux et al. 1988). The resistance of *B. cinerea* is the most widely studied, and many *B. cinerea* strains are resistant to dicarboximides (Leroux et al. 2002 ; Wang et al. 1986). Similarly, the anilinopyrimidines have proven ineffective for the control of three *B. cinerea* phenotypes sampled in French vineyards : Ani R1, Ani R2, and Ani R3 (Leroux et al. 2002). Isolates of S. minor resistant to iprodione (dicarboximide family) have also been reported in Roussillon in southern France (Davet and Martin 1993).

Insecticides, such as the carbamates, pyrethroids, and neonicotinoids, act on different targets in the nervous systems of pests. Several manifestations of resistance to these insecticides have been identified within aphid and moth populations. Four strains of *Helicoverpa armigera* among 111 sampled in Spain were highly resistant to pyrethroids (deltamethrin and lambda cyhalothrin), and 21 other strains proved to be moderately resistant (Torres-Vila et al. 2002). Many studies on the lettuce-specific aphid *N. ribisnigri* have reported its resistance to carbamates (Kift et al. 2004 ; Rufingier et al. 1999 ; Workman et al. 2004), organophosphates (Workman et al. 2004), and pyrethroids (Kift et al. 2004). However, these results are dependent on sampling location because Cuthbertson et al. (2007) found no *N. ribisnigri* resistance to pirimicarb (a carbamate) in New Zealand. The neonicotinoids are one of the newest marketed families of insecticides acting on the acetylcholine receptors of pests. Currently, the incidence of pesticide resistance is limited and localized (Jeschke and Nauen 2008 ; Nauen and Denholm 2005). Whether in the case of the fungal pathogens or the insect pests of lettuce, the development of pesticide resistance has resulted from a selection of strains suited to pesticideintensive management. This resistance can be related to various pest adaptations, such as behavioral or physiological adaptations that reduce exposure to the pesticide or limit its penetration, an increased capacity to detoxify pesticides or a conformational modification of the pesticide receptor site (Blümel et al. 2002 ; Leroux et al. 2002). Cultural practices can accelerate or delay the appearance of pesticide resistance. This resistance can be managed by (1) moderating the number of applications and doses of pesticides, (2) applying a mixture of substances with diverse actions, or (3) alternating pesticide applications among chemical families (Savary et al. 2006).

2.3 Toward innovative pest and disease management strategies that address environmental issues as well as economic and social expectations

2.3.1 One example of typical lettuce cropping systems :the production in the Mediterranean region

In the Mediterranean region, lettuces are cultivated all year round under shelter from September to April and in open fields from March to November.Water is supplied mainly by sprinklers,

but in some cases by drip irrigation. Fertilization is brought before planting, with a mixture of NPK fertilizers, to achieve a soil N content before plantation of about 100– 150 kg.ha^{-1}. Lettuces are planted mostly on plastic mulches, especially under shelter, which limits weed development. Crop density is usually between 12 and 16 plants per square meter, depending on lettuce type and commercial destination. Cultivar choices are focused on agronomic criteria, but a few growers also choose cultivars with complete resistance to *B. lactucae* (Bl :1 to Bl :28). Resistance to *N. ribisnigri* (Nr : 0) may also be a selection criterion in the case of open field crops. A few alternative techniques have been adopted recently by farmers. Solarization is generalizing, whereas biocontrol and biofumigation techniques are sometimes used. A recent survey in French lettuce fields showed that, on average, eight pesticides are applied preventively during the 60–90-day-long crop cycle. In winter, these are mainly fungicides, whereas in summer, insecticides are predominant. Beyond the case of lettuce production in the Mediterranean basin, in conventional lettuce cropping systems worldwide, pest and pathogen management is mainly achieved by preventive applications of pesticides. This crop-protection strategy provides acceptable economic performance but is not sustainable because it selects for pesticide resistance and endangers both the environment and human health.

2.3.2 No « silver bullet » but a combination of techniques

Scientists now agree that agronomic practices should be more strongly founded on biological processes occurring naturally within agroecosystems (Altieri 1989 ; Dore et al. 2011) to enhance ecosystem services, including pest and pathogen regulation. Considering the complexity of agroecosystems, there is no « silver bullet », that is, a single and sustainable method for managing pests and diseases. As mentioned above, a significant number of alternative techniques are currently available for the management of lettuce pests and diseases ; however, no single technique can provide an effective and sustainable management solution. Instead, these techniques operate at different stages of the pest cycle (invasion, development, and multiplication) and can be used in a complementary way. While many of these techniques are well defined, others require more investigation (Table 2.3). The design of crop-protection strategies less dependent on pesticides should therefore be based on the joint implementation of a consistent set of alternative techniques, each with partial effects on diseases and pests. These new protections for cropping systems could take the form of a set of decision rules that would allow customization of the practices to fit the local environment and constraints of each farm (Debaeke et al. 2009). For some lettuce pathogens and pests such as moths, there are few current alternatives to pesticides, providing little leeway to reduce pesticide use ; for other pathogens and pests, many alternative techniques are available. For example, the management of *Sclerotinia* spp. as well as many other soil-borne diseases in lettuce crops is currently based on preventive chemical control (two to three fungicide applications during the growing season) and sometimes on solarization techniques, but biological control agents, crop rotations, stimulation of plant defenses by induction or fertilization, biofumigation, and climatic management achieved by irrigation type and varietal choices could also be implemented. In addition, these techniques have complementary modes of action ; that is, solarization and crop rotation affect the survival of persistent pathogen forms, whereas the plant architecture and irrigation type affect pathogen development. Under these conditions, it appears feasible to significantly reduce fungicide applications against *Sclerotinia* spp. or to at least switch from a preventive to a curative use of the chemicals. The major efforts to reduce pesticide use in innovative cropping systems may initially focus on those pests and pathogens for which several alternative and complementary techniques are available.

2.3.3 Considering the ecological interaction within the agroecosystem

A required first step in the design of coherent combinations of techniques to manage diseases and pests is to consider all pests and pathogens that can threaten the crop and their corresponding life cycles. Many positive or negative direct interactions within this community, such as competition, facilitation, synergism, or antagonism, can affect its functioning and effects (Finney 1983). The most conspicuous illustration of these interactions is most likely the dependence of

TABLE 2.3: Techniques for the implementation of pest and disease management programs in lettuce cropping systems by available techniques and those requiring further investigation before implementation.

Type of control actions	Techniques available for implementation	Techniques requiring further investigation
Restricting the invasion of pests and pathogens	Solarization Diversification of rotation Insect proof nets Introduction of biological control agents Chemical fumigation	Biofumigation Ultraviolet protection cover Mulch Conservation biological control
Limiting pest and pathogen development and multiplication on plants	Releases of natural enemies (preventives and therapeutics) Abiotic condition improvement Genetic resistances (gene for gene) Pesticide applications	Partial genetic resistances Resistance inducers Optimization of fertilization

viruses on their vectors (e.g., aphids and thrips) to infect a plant. Such interactions are important because they can affect the success and ancillary effects of a pathogen or pest management technique. A secondary pest competing with a main one can replace it after the latter is eliminated by efficient control measures. Moreover, trophic interactions, which can be quite complicated and include intraguild predation (Polis et al. 1989) or apparent competition (Holt 1977), should be considered because of their key role in pest regulation (Matson et al. 1997 ; Van der Putten et al. 2001).

2.3.4 Prioritization of control methods

Based on compatibility and complementarity among techniques

Compatibility among techniques is decisive for the success of disease and pest management strategies. Currently, the most studied example is the compatibility between chemical and biological control. To introduce or promote auxiliary populations for pest management, it is necessary to assess non-target effects of pesticides on those populations (Blümel et al. 2002). Badawy and El-Arnaouty (1999) revealed that some organophosphorus and carbamate insecticides had, respectively, a high and moderate effect on the survival of eggs and larvae of *Chrysoperla carnea*. Insecticides may have not only direct toxic effects on the natural enemies of pests but also indirect non-target effects due to consumption of poisoned prey by predators (Walker et al. 2007 ; Badawy and El-Arnaouty 1999). Although the selectivity of active substances is increasingly considered for pesticide registration (Palumbo and Castle 2009), reports of non-target effects are common. To be relevant, the evaluation of side effects should be performed in conditions close to those of the field. The effects on *Coniothyrium minitans* of all the pesticides applied in lettuce crops in the UK have been investigated in this manner (Budge and Whipps 2001). Iprodione strongly affected the germination and mycelial growth of *C. minitans* in an agar plate test but not when the experiment was performed in a soil tray. Additionally, the joint application of a reduced rate of iprodione and a biological control agent (*C. minitans*) in a field study resulted in efficient control of *S. sclerotiorum*. Henderson et al. (2009) have shown that chemical compounds released by biofumigation with mustard can also affect the efficacy of biological control of plant parasitic nematodes by entomopathogenic nematodes such as *Steinernema felsiae*.

There is sometimes a conflict among biological control agents. Hindayana et al. (2001) highlighted the intraguild predation among aphidophagous predators, including *C. carnea* and *Episyrphus balteatus*. *C. carnea* could be a predator for *E. balteatus* and vice versa depending on

their developmental stages. This interaction was exacerbated by the lack of more appropriate prey (aphids). Such results have to be considered for the implementation of efficient biological control.

Beyond compatibility, the design of innovative cropping systems aims to combine techniques that have complementary effects on diseases and pest. Such complementarity results in increased control of pests and diseases through the additional effects of the partial efficacies of each technique, and it is based both on the compatibility among techniques and complementarities of their modes of action. The techniques can be used separately at different times in the life cycle of the target pest and pathogen or simultaneously. Spadaro and Gullino (2005) have reviewed possible complementary techniques to be used in combination with biological control agents against soil-borne pathogens. These techniques include, for example, the use of other biological control agents, reduced applications of pesticides, or soil disinfestation methods. According to Melo et al. (2011), an approach for improving the efficiency of *C. minitans* is the development of mutants with greater capacities for parasitism, growth, and resistance to fungicides. In this case, biological control is associated with genetic modifications. In another approach, Van Beneden et al. (2010) have demonstrated the possibility of increasing *C. minitans* parasitism of sclerotia (*S. sclerotiorum*) by the incorporation into the soil of Kraft pine lignin, which is believed to promote the development of decomposers of the sclerotial walls. Currently, solarization is most likely the technique most commonly combined with others for the management of soil pests. To improve its effects on soil-borne pests and pathogens, solarization may be associated with the application of bio-control agents, chemical pesticides, or organic amendments with disinfectant properties (Gil et al. 2009 ; Stapleton and Devay 1983).

The complementarity of pest management techniques has also been investigated for the control of lettuce aphids. Fagan et al. (2010) assessed the combined effect of chemical control, which consisted in drenching seedlings with a systemic insecticide (imidacloprid), and biological control with natural predators of aphids (*N. ribisnigri*). They observed complete control of aphid populations in the mid-summer period. Similarly, Parker et al. (2002) have investigated the efficacy of integrated pest management programs against lettuce aphids and showed that varietal resistance, the introduction of entomopathogenic fungi, and seedling drenches with insecticide help reduce the number of foliar applications of fungicides during the crop cycle.

Based on their impact on agroecosystem services

In addition to the ecosystem service of pest and pathogen regulation, the productivity and sustainability of agroecosystems are based on their ability to provide other ecological services such as nutrient cycling or pollination. Consequently, external inputs and other compensatory practices that disturb the ecosystem should be overcome. Ecosystem services rely on beneficial organisms in the agroecosystem ; therefore, the alternative techniques used to control pests and diseases should promote ecosystem biodiversity to enhance associated services. For example, to increase soil fertility in agroecosystems, the alternative techniques for managing pests and diseases should favor organisms that support nutrient cycling (e.g., organic matter decomposers and nitrogen-fixing bacteria). The techniques that could negatively affect these beneficial organisms and thereby reduce ecosystem services should be used only when less disruptive alternatives are unavailable (Lewis et al. 1997). The consideration of ecological interactions within the agroecosystem, the compatibility among management techniques, and the selection of techniques with low impact on agroecosystem services appear to be key factors for achieving efficiency and sustainability of cropping systems. The performances of such innovative cropping systems need to be assessed.

2.3.5 Performance assessments

Cropping system assessment is critical to ascertain whether environmental, economic, and social expectations are attained. Performance assessments of innovative cropping systems are conditioned by the definition of goals. For each goal, one or more performance indicators can be employed. Thus, indicators such as the amount of active ingredient applied per hectare,

the treatment frequency index, or the environmental impact quotient (Kovach et al. 1992) may be used to assess the ability of the system to reduce pesticide applications. Agri-environmental indicators (e.g., biodiversity, soil properties, and water quality) can be used to estimate the effect of the joint implementation of agricultural practices on the biotic and abiotic components of the agroecosystem and consequently on the ecosystem services (Dale and Polasky 2007 ; Bockstaller et al. 1997). Others such as gross margin and yield are indicators of economic performance and can help determine whether a reduced reliance on phytosanitary products affects farm profitability. Finally, working time, the total number of operations, and the operation costs are indicators of social performance (Lancon et al. 2007). The agroecosystem performances can be assessed one by one or with a multiobjective approach of agricultural sustainability, which can be translated into environmental, economic, and social goals (Hansen 1996 ; Lichtfouse et al. 2009). These objectives can sometimes be antagonistic. Therefore, multicriteria evaluations are helpful to design and assess innovative cropping systems (Ould-Sidi and Lescourret 2011). Multicriteria assessment tools have been designed for various crops to estimate qualitatively and *ex-ante* (i.e., before system implementation) the sustainability of cropping system prototypes (Pelzer et al. 2012 ; Sadok et al. 2009). However, these tools have not yet been adapted for the assessment of lettuce cropping systems.

2.4 Conclusion

For decades, major advances have been made in the development of alternatives to pesticide application for the protection of lettuce crops. They include the manipulation of the biotic (e.g., diversification of rotations and the introduction of plant diversity) and abiotic environment (e.g., insect-proof nets and ultraviolet protection covers), methods to increase plant defenses (e.g., genetic resistance, inducers, and defenses), and biocide effects on pathogens and pests (e.g., solarization and the introduction of biological control agents). However, as for other crops, there is no « silver bullet » to manage sustainably the populations of lettuce pests and pathogens, and consequently, the use of multiple alternative techniques with partial and complementary effects seems to be the best option to reduce phytosanitary inputs. In the current state of knowledge, the design of innovative cropping systems, adapted to the epidemic risks, appears feasible and should help to reduce the use of phytosanitary products. In the Mediterranean region, the main sources of epidemic risk for lettuce crops in winter are fungal diseases (see Table 2.1). In this case, solarization or introduction of biological control agents may be considered to reduce the soil primary inoculum. Then, the use of genetic resistance, the implementation of a drip irrigation, and the reduction of nitrogen input can create an unfavorable environment for pathogen development. The risk of infestation by aphids could be managed through the preventive introduction of biological control agents. In contrast, the implementation of insect-proof nets is not recommended since it may increase the humidity inside the greenhouse, which is generally favorable to pathogens.

This review provides a reflective tool for the design of such disease and pest management strategies. The approach proposed for managing pests and pathogens of lettuce goes beyond the integrated pest management approach because it advocates a prioritization of practices based on their effects on ecosystem services. As previously suggested by Lewis et al. (1997), techniques with biocide effects that disrupt the agroecosystem should be used as a last resort to promote the natural regulation of pest and pathogen populations, mediated by multitrophic interactions.

Furthermore, the absence of performance data for the joint use of alternative techniques is a severe hindrance to the adoption by growers of novel control strategies associating complementary techniques. So, studies should next focus on the implementation and assessment of cropping system prototypes that are based on available knowledge and designed according to the approach proposed above. Future research should also address the knowledge gaps identified in this review.

2.5 Acknowledgments

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Deuxième partie

APPROCHE ANALYTIQUE : Approfondissement des connaissances sur les techniques alternatives visant à contrôler les pathogènes et ravageurs de la laitue

Chapitre 3

Susceptibility of lettuce to *Botrytis cinerea* and *Sclerotinia sclerotiorum* : Influence of genotype and sugar content [1]

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Abstract

Botrytis cinerea and *Sclerotinia sclerotiorum* are two necrotrophic fungi causing major losses in lettuce crops. Both pathogens are generally managed with preventative applications of pesticides, but alternatives to chemical treatments are required. Resistance to the two fungi might be conferred by suitable plant growth habit or tissue composition. However it is unknown whether tissue-based- resistance to both fungi could be found in a single cultivar. It has been shown that environmental conditions and the resulting plant metabolic status can affect its susceptibility to both pathogens, but the extent of the interaction between genotype and environment is also unknown. To characterize tissue-based- resistance, 9 accessions of lettuce were tested in four independent experiments with inoculations of *B. cinerea* and *S. sclerotiorum* on detached leaves. The lesion expansion rates were up to 7 and 4 times lower in the most resistant accessions than in the most susceptible ones, for *B. cinerea* and *S. sclerotiorum* respectively. The level of susceptibility to *B. cinerea* and *S. sclerotiorum* was also influenced by leaf sugar content. Fructose content was positively correlated to *B. cinerea* lesion expansion rate and sucrose content was negatively correlated to *S. sclerotiorum* and *B. cinerea* lesion expansion rates. The susceptibility to *S. sclerotiorum* of 6 accessions was further examined at crop level. Results were in agreement with tests performed on detached leaves, suggesting that a phenotyping method based on detached leaves could be reliable to predict field resistance. They also suggest that breeding efforts should focus on accessions showing high tissue-based- resistance, while both genotype and growing conditions should be adapted to achieve a low fructose : sucrose ratio.

Keywords

Necrotrophic pathogens, lettuce, tissue based- resistance, sugar, crop protection

1. Ce chapitre à été soumis à la revue *Plant Disease*

3.1 Introduction

Botrytis cinerea (Pers.) and *Sclerotinia sclerotiorum* (Lib.) de Bary are two ubiquitous necrotrophic plant pathogens with wide host ranges (Boland and Hall, 1994 ; Williamson et al., 2007). Both attack lettuce (*Lactuca sativa* L.) and can induce fairly similar symptoms so that they were often mistaken for one another in lettuce crops until 1900 (Smith, 1900 ; Purdy, 1979). The two pathogens have similar infection strategies (Prins et al., 2000), with many common pathogenesis-related genes (Amselem et al., 2011). They both secrete proteins and toxins causing cell wall degradation and cell leakage, such as cutinases, pectinases and oxalic acid (Bolton et al., 2006 ; Prins et al., 2000), which allow them to proliferate in macerated tissues. Due to their ability to produce sclerotia, ensuring them long term survival, they can persist in the field and infect plants in favorable conditions. Due to its high relative humidity requirement ($> 95\%$), *B. cinerea* is mainly observed in protected lettuce crops while S. sclerotiorum can also cause significant damage in open fields (Blancard et al., 2006).

In conventional lettuce cropping systems, *B. cinerea* and *S. sclerotiorum* are mainly managed by fungicide applications. The same active substances are used to control either pathogen, and resistance to fungicides has been observed, both within *B. cinerea* (Leroux et al., 2002, Wang et al., 1986) and *S. sclerotiorum* populations (Gossen et al., 2001). Therefore, replacement of pesticides by alternative techniques should ideally allow control of both *B. cinerea* and *S. sclerotiorum* when they both threaten, such as in protected lettuce crops. Several alternative techniques with partial effects on *B. cinerea* and/or *S. sclerotiorum* are currently available, including biological control (De Meyer et al. ; 1998, Zeng et al., 2012), soil disinfestation methods (Patrício et al., 2006 ; Kurt et al., 2011) and methods that create unfavorable conditions for disease development, such as drip irrigation and reduced planting density (Maas et al., 2006 ; Wu and Subbarao, 2003). Planting resistant varieties could also be a part of a *B. cinerea* and *S. sclerotiorum* management strategy for several plant species (Bolton et al., 2006 ; Kurle et al., 2001). Huang et al. (2002) showed a positive correlation between the size of lesions caused by *B. cinerea* and by *S. sclerotiorum* on dry beans. These findings indicate that mechanisms of plant resistance to both pathogens could be overlapping. Currently, heritable resistance has been identified in several plant species, including tomato and chickpea for *B. cinerea* (Finkers et al., 2007 ; Anuradha et al., 2011) and soybean, sunflower and rapeseed for *S. sclerotiorum* (Arahana et al., 2001 ; Mestries et al., 1998 ; Zhao and Meng, 2003).

In lettuce accessions, different levels of susceptibility to *S. sclerotiorum* were observed (Madjid et al., 1983). The low susceptibility levels observed for certain accessions was defined as "slow-dying resistance" by Subbarao (1998) since it results from a slowdown in the progression of disease. This resistance may have different backgrounds and could depend on tissue composition and lettuce growth habit (Hayes et al., 2010). Several studies on lettuce and other species have reported that plant growth habit could be correlated to a lower disease incidence (Haware et al., 1997, Sherf and MacNab, 1986, Newton and Sequeira, 1972). For example, an upright plant architecture of the lettuce plant can foster airflow under the canopy, thereby allowing disease escape by the creation of a dry microclimate, unfavorable for the infection process (Newton and Sequeira, 1972). However, recent work has shown that low susceptibility to *Sclerotinia* sp. could also be independent of the plant growth habit, and thus be based on tissue composition (Hayes et al., 2011 ; Hayes et al., 2010). This hypothesis is strongly supported by the fact that very similar lettuce types can have different levels of susceptibility to *Sclerotinia* sp. (Hayes et al., 2011). The introgression of tissue-based- resistance would be more compatible with market expectations since lettuce diversification requires the co-existence of multiple lettuce growth habits. However, protocols are needed to test specifically for tissue-based- resistance, since observations of disease susceptibility on entire plants can be confusing. Several authors have investigated resistance of lettuce to *S. sclerotiorum* in greenhouse experiments (Hayes et al., 2010 ; Madjid et al., 1983 ; Whipps et al., 2002) by spray-inoculating the whole plants with ascospore suspensions. This type of inoculation has the advantage of overriding the possible impact of lettuce growth habit on tissue colonization by the pathogen. However, it does not remove the dependence on a possible impact of plant growth habit on the initial infection process, through the establishment of a humid microclimate in the foliage of certain lettuce types (Newton and Sequeira, 1972). Therefore, it

Type	Accessions	Origin (registration year, use)	Experiments
Red Batavia	Blonde à bord rouge	Public cultivar (field)	E1 to E6
	Gloire du Dauphiné	Public cultivar (field)	E1 to E6
	Carlita	Vilmorin (1989, greenhouse)	E1 to E6
Green Batavia	Delsay	Seminis (2001, field)	E1 to E6
	Lasydo	Syngenta seeds (2012, greenhouse)	E1 to E6
	Palomis	Gautier Semences (2007, greenhouse)	E1 to E6
	Reine des glaces	Public cultivar (field)	E1 to E4
Green Butterhead	Armonica	Rijk Zwaan (2006, greenhouse)	E1 to E4
	Faustina	Rijk Zwaan (2004, field)	E1 to E4

TABLE 3.1: Horticultural type, origin and use of lettuce accessions in the different experiments.

would be interesting to identify unambiguously tissue -based resistance in laboratory conditions, e.g. on detached leaves, prior to the evaluation of entire plant susceptibility. Since the two fungi are managed with similar methods, in particular fungicides, valuable information could arise from the appraisal of common resistance in the same genotype. To our knowledge, no information is available on the varietal differences of lettuce resistance to *B. cinerea*.

The plant content of primary metabolites, especially soluble sugars and nitrate, have been correlated to lettuce (cv. Faustina) susceptibility to both *B. cinerea* and *S. sclerotiorum* (Lecompte et al., 2013). Thus we hypothesized that sugar metabolism could be involved in tissue-based-resistance to *B. cinerea* and *S. sclerotiorum*. Since sugar content is largely dependent on the environment, the resistance conferred by the metabolic status could either be the consequence of a genotype :environment interaction or be independent of the genotype.

In the present study, experiments were conducted on detached leaves in controlled conditions to assess the resistance of lettuce accessions to *B. cinerea* and *S. sclerotiorum* independently of lettuce growth habits. The resistance described here is typically a "slow-dying resistance". The objective of this work was (i) to identify genetic diversity for such resistance to *B. cinerea* and *S. sclerotiorum* among lettuce accessions and assess its potential value in breeding, (ii) to determine whether slow-dying resistance to *B. cinerea* and *S. sclerotiorum* can exist in the same genotype, (iii) to determine the dependence between results obtained on detached leaves and at crop level, and (iv) to determine the relationship between leaf metabolite content and susceptibility to *B. cinerea* and *S. sclerotiorum*.

3.2 Material and Methods

3.2.1 Plant material

Nine accessions of *L. sativa* (Table 3.1) were used in this study. They were chosen from an initial pool of 21 accessions and represented the range of resistance observed in this initial pool during preliminary tests. Seeds originated from the CRBLég (INRA-GAFL) collection (7 cultivars) or were kindly provided by the seed companies Gautier Semences (cv Palomis) and Syngenta seeds (cv Lasydo). Green and red batavia and green butterhead accessions were selected from the CRBLég collection, assuming that the use of different horticultural types would increase the genetic diversity of the sample, or based on published results on their level of susceptibility to *S. sclerotiorum* (Hayes et al., 2010 ; Elia and Piglionica, 1964). Three of the 9 tested accessions were old public domain cultivars (Table 1), while the other 6 were modern cultivars, which have resulted from selection schemes mostly focused on agronomic qualities and resistance to downy mildew caused by *Bremia lactucae* (Regel). Four of these 6 cultivars, still registered in Europe, were currently used by growers (Lasydo, Palomis, Armonica and Faustina).

3.2.2 Detached leaf experiments

The data collected from detached leaf experiments were used to explore the stability of assessments in different experimental conditions (mostly weather during plant production and pathogen strains), the correlation between susceptibilities to *B. cinerea* and *S. sclerotiorum* and to investigate the effect of the leaf metabolic status on susceptibility, as described below.

Experimental design and plant production

Four independent experiments were performed on detached leaves (E1, E2, E3, E4) (Table 3.2). In each experiment, 5 plants per accession were tested using 2 detached leaves per plant (one for each pathogen tested). Seeds were sown in trays containing a sowing mix of peat and fine coco fiber ('seedling substrate', Klasmann-Deilmann GmbH, Geeste, Germany) and placed in a nursery greenhouse. Fifteen days after sowing, the seedlings were transferred to individual pots containing a bedding plant substrate ('TS3 bedding plant substrate', Klasmann-Deilmann GmbH, Geeste, Germany) and placed in a second greenhouse dedicated to the experiments in a complete randomized block design.

Experiment type	Strain inoculated	Experiment number	Sowing date	Inoculation date
Detached leaf experiments	*B. cinerea* BC1 and *S. sclerotiorum* SS1	E1	1/24/2012	3/12/2012
		E2	2/21/2012	4/10/2012
	B. cinerea SAS405 and *S. sclerotiorum* SS2	E3	5/6/2013	6/17/2013
		E4	5/13/2013	6/25/2013
Entire plant experiments	*S. sclerotiorum* SS2	E5	9/24/2013	12/18/2013
		E6	1/9/2014	3/20/2014

TABLE 3.2: Experimental designs used to screen 9 lettuce accessions for resistance to *Botrytis cinerea* and *Sclerotinia sclerotiorum*.

Assessment of resistance to *B. cinerea* and *S. sclerotiorum*

Six to seven weeks after sowing, medium-sized leaves were excised and placed on sheets of wet paper in clear polystyrene boxes. Two strains of *B. cinerea* (BC1 and SAS405) and two of *S. sclerotiorum* (SS1 and SS2), used routinely in the laboratory, were used to assess the level of susceptibility of the accessions. The primary inoculum for both pathogens was produced in Petri dishes on potato dextrose agar incubated at 21 ° C under cool white fluorescent light (14 h photoperiod ; 114 μmol.m^{-2}.s^{-1}). After 3 days of culture, mycelial plugs were excised from the growing margin of the colony with a cork borer and placed in the center of each leaf, on the adaxial midrib, taking care that the side bearing mycelium was put in contact with the plant tissue. The polystyrene boxes were then randomly placed to incubate in a growth chamber at 21 ° C with a 12 h photoperiod. The lesion size was assessed 2, 3 and 4 days after inoculation. For leaves inoculated with *S. sclerotiorum* no assessment was carried out at 4 days after inoculation as the lesions had reached the margin of the leaves for most accessions. Each leaf was photographed over a blue background and the image analysis software Assess 2.0 (APS Press, St Paul, MN, USA) was used to quantify lesion size (in mm^2). For each inoculation point, the rate of lesion expansion was calculated as the average daily increase in lesion size between the day of symptom appearance on the leaf and the last day of measurement. Resistance was thus defined as the ability of the plants to slow the progression of lesions. The rate of lesion expansion (L) was calculated for each detached leaf as :

$$L_{B.\ cinerea} = (S4 - S2)/2 \text{ and } L_{S.\ sclerotiorum} = S3 - S2$$

where S2, S3 and S4 are the lesion size (mm^2) 2, 3 and 4 days after inoculation respectively.

Analysis of plant metabolites

In each experiment, 2 additional leaves of the same age were excised from each plant. The leaves were immediately placed in liquid nitrogen and then stored at 20 ° C. Sub-samples of dry material were calcined at 400 ° C for 12 h and then mineralized in boiling HNO_3. Nitrate content was measured with a NO_3/NO_2 analyzer (5000 FIAstar). Main soluble sugar contents (glucose, fructose and sucrose) were assessed in a microplate reader (MICROSKAN Ascent - Thermo scientific) following an enzymatic determination method described by Gomez et al. (2007).

3.2.3 Crop level experiments

Two field experiments (E5 and E6) were performed to examine the relationship between resistance to *S. sclerotiorum* in detached leaf experiments and in entire plants. Field resistance to *S. sclerotiorum* only was assessed, since repeated inoculation trials with *B. cinerea* in the field did not result in sufficient and uniform infection rates, regardless of the accession.

Experimental design

Each experiment was carried out with 6 of the Batavia accessions ('Reine des glaces' not included) used from E1 to E4 (Table 3.1). On September 24^{th} 2013 and January 9^{th} 2014, seedlings were raised in a nursery greenhouse in seed trays, each containing 120 individual plugs of peat seedling substrate. The four-week-old seedlings were then transplanted into two adjacent 8 x 16 m unheated plastic tunnels. These tunnels were selected on the basis of previous occurrence of *Sclerotinia* lettuce drop. The lettuces were planted in a complete randomized block design with six blocks per tunnel (three blocks located on each side of the central path in the tunnel). Each block comprised six randomized 1.80 x 2 m plots (one per accession) of 30 plants. In total, each accession was thus represented by 360 plants in each experiment. To compensate for possible insufficient or unevenly-distributed natural inoculum, half of the plants in each tunnel (three of the six blocks per tunnel, located at the East side in E5 and at the West side in E6) were inoculated with a suspension of mycelial fragments produced as follows.

Evaluation of resistance to *S. sclerotiorum*

Strain SS2 of *S. sclerotiorum* was grown on potato dextrose agar medium at 21 ° C for 3 days as described above for detached leaf experiments. For each experiment, 50 Petri dishes (90 mm diameter) were used. The content of the plates (agar included) was cut in pieces and ground for 15 s in a Waring blender. The mixture was then filtered through a layer of cheese cloth (to remove insufficiently-ground pieces of agar) and adjusted to a volume of 5.4 liter with sterile water. The inoculum was then applied to the plants, using a backpack sprayer. Each plant received on average 5 ml of mycelial suspension. Approximately one month after inoculation, the number of infected plants per plot and the severity of disease were recorded for each plant. Severity scores were 0= no symptoms ; 1= 3 or less leaves with symptoms ; 2= more than 3 leaves with symptoms ; 3= complete lettuce drop.

3.2.4 Data analysis

All the statistical analyses were performed using the R software (R-Core-Team, 2013).

Analysis of accession effect on lesion expansion in detached leaf experiments

The effect of accession on the rate of lesion expansion was evaluated with an analysis of variance (ANOVA) for each detached leaf experiment. Significant differences of the rate of lesion expansion among accessions were examined with Tukey's mean comparison test, which was also used to sort accessions for their overall susceptibility (for E1 to E4) to each pathogen. Correlation between $L_{B.\ cinerea}$ and $L_{S.\ sclerotiorum}$ of each individual was assessed with Pearson's correlation coefficient.

Identification of metabolite contents that explained lesion expansion in detached leaf experiments and variability among experiments and accessions

Since the sugar and nitrate contents are likely to be correlated in plants, a statistical approach was used to identify the independent effects of metabolites on lesion expansion rates. In a multiple linear model, the type 1 errors indicating the sums of squares attributable to each explanatory variable are conditioned by those of the variables placed forward in the model. Thus, if the variable placed in the last position proves to be statistically significant, this reveals that at least a part of its effect on the response variable is independent of the other variables. Based on these considerations, linear model analyses (with pooled data from the four experiments) were used, with all possible combinations of explanatory variable orders, to identify the metabolite variables (nitrate, fructose, glucose and sucrose) that, independently of each other, affect the lesion expansion rate for each pathogen. For each pathogen, linear models were then designed with the rate of lesion expansion as response variable and the metabolite variables that, independently of the others affect lesion expansion rate, as explanatory variables. The rates of lesion expansion were square-root-transformed prior to analysis to normalize the residuals of the models. Then, for the metabolite variables that independently of the others affect the rate of lesion expansion, we identified the effect of accession and experiment on their variability, using ANOVA with square-root-transformed variables.

Overall model

Then, to investigate the effect of the interaction between accession and metabolite content on lettuce susceptibility, a linear mixed model was designed for each pathogen with $L_{B.\ cinerea}$ and $L_{S.\ sclerotiorum}$ as response variables and with accession, the metabolites that best explained the rate of lesion expansion for each pathogen, and their interaction as fixed effects, and with experiment as a random effect. The rates of lesion expansion were square-root-transformed prior to analysis to normalize the residuals of the models.

Analysis of accession effect on lesion expansion in crop level experiments

For inoculated and naturally infested plots, disease incidence data were analyzed separately using a mixed generalized linear model with a binomial error and with accession as fixed variable and experiment as random variable. Differences of disease incidence among accessions were studied with Tukey's mean comparison test.

3.3 Results

3.3.1 Effect of accession on lesion expansion in detached leaf experiments

The average lesion expansion rates of both *S. sclerotiorum* and *B. cinerea* varied greatly between experiments (Table 3.3). Lesions caused by *S. sclerotiorum* generally expanded faster than those caused by *B. cinerea*, except in E3.

The highest rates of lesion expansion were observed in E2 and E4 and the lowest rates in E3 and E1 for *S. sclerotiorum* and *B. cinerea* respectively. A weak but significant correlation between susceptibility to the two pathogens was observed (Pearson coef. : 0.26 ; P= 0.002). Within each experiment, significant differences in lesion expansion rate among accessions were observed in E1 and E2 for both *B. cinerea* and *S. sclerotiorum*. In E1, the rate of *B. cinerea* lesion expansion was 7 times faster for 'Lasydo' and 'Palomis' than for 'Gloire du Dauphiné'. In E2, it was only 2.5 times faster. For *S. sclerotiorum*, the rates of lesion expansion were 4.7 and 2.3 times faster for 'Armonica' than for 'Gloire du Dauphiné' in E1 and E2 respectively. Although the severity of symptoms varied widely, the ranking of varieties for their slow-dying resistance to each pathogen was generally consistent among experiments except for Lasydo. The average susceptibility of accessions and their overall ranking are shown in Figure 3.1. 'Gloire du Dauphiné' displayed

S. sclerotiorum					*B. cinerea*				
Lettuce Accession	Strain SS1		Strain SS2		Lettuce Accession	Strain BC1		Strain SAS405	
	E1	E2	E3	E4		E1	E2	E3	E4
Gloire du Dauphiné	473 a	1383 a	154	979	Gloire du Dauphiné	44 a	120 a	160	437
Lasydo	521 a	2504 cde	28	1130	Delsay	112 a	138 a	146	517
Blonde à bord rouge	857 ab	1515 ab	242	719	Faustina	122 a	158 ab	392	538
Delsay	977 ab	1871 abc	314	844	Reine des glaces	123 a	148 ab	123	562
Reine des glaces	1124 ab	2298 bcd	158	1081	Blonde à bord rouge	213 b	113 a	150	477
Palomis	1128 ab	2025 abcd	77	1283	Armonica	237 b	280 c	406	704
Faustina	1236 ab	2445 cde	38	1259	Carlita	275 bc	257 bc	222	545
Carlita	1571 ab	2768 de	115	840	Palomis	308 bc	319 c	183	654
Armonica	2227 b	3206 e	598	1496	Lasydo	311 bc	305 c	140	510
Average	1138	2224	193	1070	Average	192	204	193	549
F-value	3.06	9.46	2.62	1.99	F-value	10.17	11.84	2.04	2.28
p-value	0.0106	<0.0001	0.0548	0.1074	p-value	<0.0001	<0.0001	0.1158	0.0694

TABLE 3.3: Rate of lesion expansion ($mm^2.day^{-1}$) of *Sclerotinia sclerotiorum* and *Botrytis cinerea* strains on detached leaves of lettuce accessions in experiment E1 to E4 (means of 5 plants x 1 leaf). F-value and p-value are the results of ANOVA. Letters give the differences among accessions ($P < 0.05$) according to Tukey's mean comparison tests.

the lowest lesion expansion rates to both *B. cinerea* and *S. sclerotiorum*. 'Blonde à bord rouge' and 'Delsay' were among the least susceptible accessions too, while 'Armonica', 'Palomis' and 'Carlita' displayed high lesion expansion rates with both pathogens.

3.3.2 Effect of metabolite content on lesion expansion in detached leaf experiments

The metabolite contents that were selected to be included in the linear model because of their effect on the rate of lesion expansion, independently of the other variables, were fructose and sucrose for *B. cinerea* and sucrose for *S. sclerotiorum*. With these explanatory independent variables, a linear model was fitted to assess the relationship between lesion expansion and leaf metabolic content for each pathogen. Both the fructose (F-value= 41.606 ; 1 df ; $P < 0.0001$) and the sucrose contents (F= 12.559 ; 1 df ; $P < 0.001$) for *B. cinerea* (R^2 : 0.2895) and sucrose (F= 29.161 ; 1df ; $P < 0.0001$) for *S. sclerotiorum* (R^2 : 0.1803) showed significant effects on lesion expansion rates. Fructose content was positively correlated with *B. cinerea* (Figure 3.2a) lesions while sucrose content was negatively correlated with *S. sclerotiorum* (Figure 3.2b) and *B. cinerea* lesions (data not shown). Differences in fructose contents observed among individuals arose both from accession (F= 7.4618 ; 8 df ; $P < 0.0001$), experiment (F=115.9525 ; 3 df ; $P < 0.0001$) and their interaction (F= 5.2280 ; 24 df ; $P < 0.0001$). Differences in sucrose contents among individuals also came both from accession (F= 12.631 ; 8 df ; $P < 0.0001$), experiment (F= 145.255 ; 3 df ; $P < 0.0001$) and their interaction (F= 8.195 ; 24 df ; $P < 0.0001$). However, most of the variability of sugar content accounted for differences between experiments (Figure 3.3a). Fructose content varied from 36.4 $mg.g^{-1}$ fresh weight (fw) in E1 to 71.6 $mg.g^{-1}$ fw in E4 and sucrose from 5.9 $mg.g^{-1}$ fw in E2 to 25.8 $mg.g^{-1}$ fw in E3. The range of sugar content among accessions was lower, from 40.51 to 55.28 $mg.g^{-1}$ fresh weight for fructose and from 9.49 to 21.89 $mg.g^{-1}$ fresh weight for sucrose (Figure 3.3b).

3.3.3 Relationship between metabolite content and inter-experimental variability

A mixed model with the metabolite contents that best explained susceptibility (fructose and sucrose for *B. cinerea* and sucrose for *S. sclerotiorum*), accession and their interaction as fixed effects and experiment as random effect was then tested for both pathogens. For *B. cinerea*, a

FIGURE 3.1: Lesion expansion rates (L) (means of 1 leaf x 5 plants x 4 detached leaf experiments - $mm^2.day^{-1}$) on detached medium-sized leaves of 9 lettuce accessions inoculated with mycelial plugs of *Botrytis cinerea* (**A**) or *Sclerotinia sclerotiorum* (**B**). Letters indicate significant differences among accessions ($P < 0.05$; Tukey's mean comparison test).

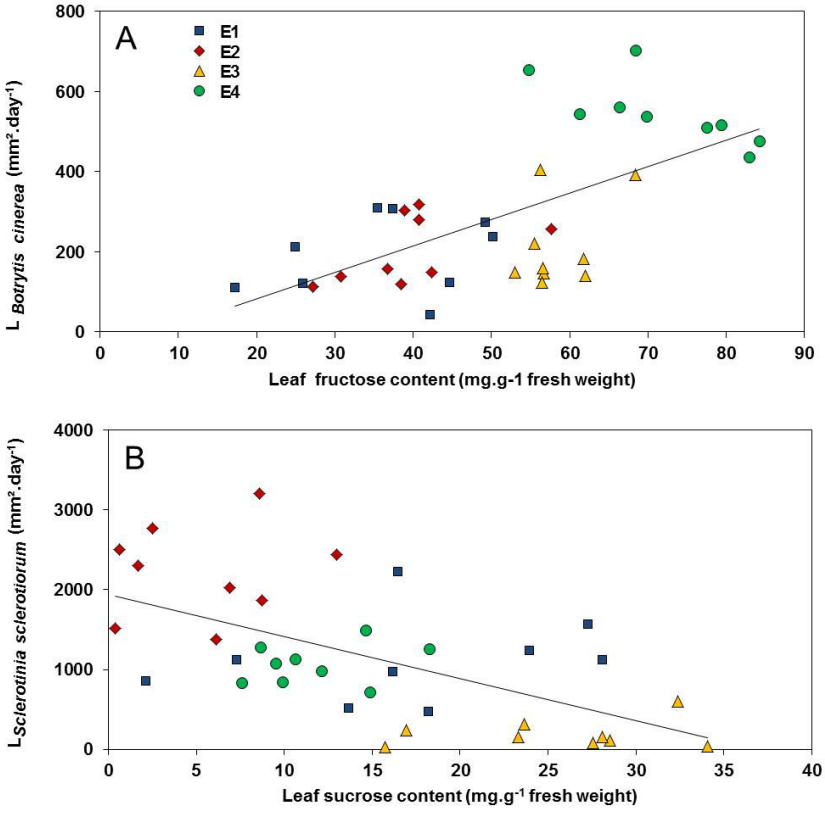

FIGURE 3.2: Relationship between the rates of lesion expansion (L) ($mm^2.day^{-1}$) on detached medium-sized leaves of 9 lettuce accessions inoculated with mycelial plugs of *Botrytis cinerea* (**A**) and *Sclerotinia sclerotiorum* (**B**) and fructose and sucrose contents of the leaves in 4 experiments (E1 to E4). Each symbol represents the mean of 5 individuals of the same accession. Black lines represent linear trends.

Lettuce accession	Lettuce infected in inoculated plots (%)[x]	Lettuce infected in naturally infested plots (%)[y]
Blonde à bord rouge	64.2	11.7 a
Delsay	59.7	19.7 b
Lasydo	67.5	28.1 c
Gloire du Dauphiné	71.9	30.8 d
Palomis	70.0	41.7 e
Carlita	85.8	53.1 f

TABLE 3.4: Incidence of lettuce drop caused by *Sclerotinia sclerotiorum* on 6 lettuce accessions in crop level experiments with inoculated and naturally infested plots (data show means of 6 blocks x 2 field experiments). [x] No significant difference between accessions (P >0.05). [x] Different letters indicate statistically significant differences between accessions (P < 0.05) according to Tukey's mean comparison test.

significant effect of accession (F= 3.5721 ; 8 df ; P= 0.001115) was observed, but there was no effect of fructose (F= 0.5954 ; 1 df ; P= 0.442149) or sucrose contents (F= 0.0206 ; 1 df ; P= 0.886109), nor of the interactions between accession and fructose content (F= 1.2547 ; 1 df ; P= 0.275982) or between accession and sucrose (F= 1.0674 ; 1 df ; P= 0.392162). For *S. sclerotiorum* lesion expansion, there was a significant effect of accession (F= 3.4848 ; 8 df ; P= 0.001293), but not of sucrose content (F= 0.1520 ; 1 df ; P= 0.697411) nor of the interaction (F= 1.0915 ; 8 df ; P= 0.374733). Since a random effect of the experiment was taken into account in the models, the absence of effect of the fructose and sucrose contents on the rates of lesion expansion confirmed that most of the sugar content effect was confounded with the inter-experimental variability. Both the high variability of sugar content among experiments and the mixed model analysis showed that the susceptibility associated to sugar content and the genotype-based resistance were independent.

3.3.4 Effect of accession on lesion expansion in crop level experiments

In field experiments, significant differences of *S. sclerotiorum* incidence among accessions were observed in naturally infested plots but not in inoculated ones (Table 3.4). On average, 69.9% of lettuces were infected in inoculated plots while only 30.8% were in naturally infested plots. From the most resistant to the most susceptible accession, the ranking of lettuce accessions for their susceptibility to *S. sclerotiorum* in the naturally infested plots, was 'Blonde à bord rouge', 'Delsay', 'Lasydo', 'Gloire du Dauphiné', 'Palomis' and 'Carlita' (Table 3.4). Except for 'Gloire du Dauphiné', which was the most resistant accession in detached leaf experiments, this ranking, obtained at crop level was consistent with that obtained in detached leaf experiments.

3.4 Discussion

Lettuce resistance to necrotrophic pathogens could result from both plant growth habit and tissue composition of the leaves. In our experiments, we investigated the resistance to *B. cinerea* and *S. sclerotiorum* not related to lettuce growth habit using detached leaf experiments. None of the 9 accessions of *L. sativa* displayed complete resistance to *B. cinerea* or *S. sclerotiorum* but, despite the limited number of accessions used, the range of variation of "slow-dying resistance" observed among accessions was relatively high. Indeed, in the most discriminating experiment, levels of "slow-dying resistance" were up to 7 and 4 times higher in the most resistant accessions than in the most susceptible ones for *B. cinerea* and *S. sclerotiorum* respectively. To our knowledge, this is the first report of variations of susceptibility to *B. cinerea* among lettuce accessions. For *S. sclerotiorum*, our results are consistent with previous investigations which showed resis-

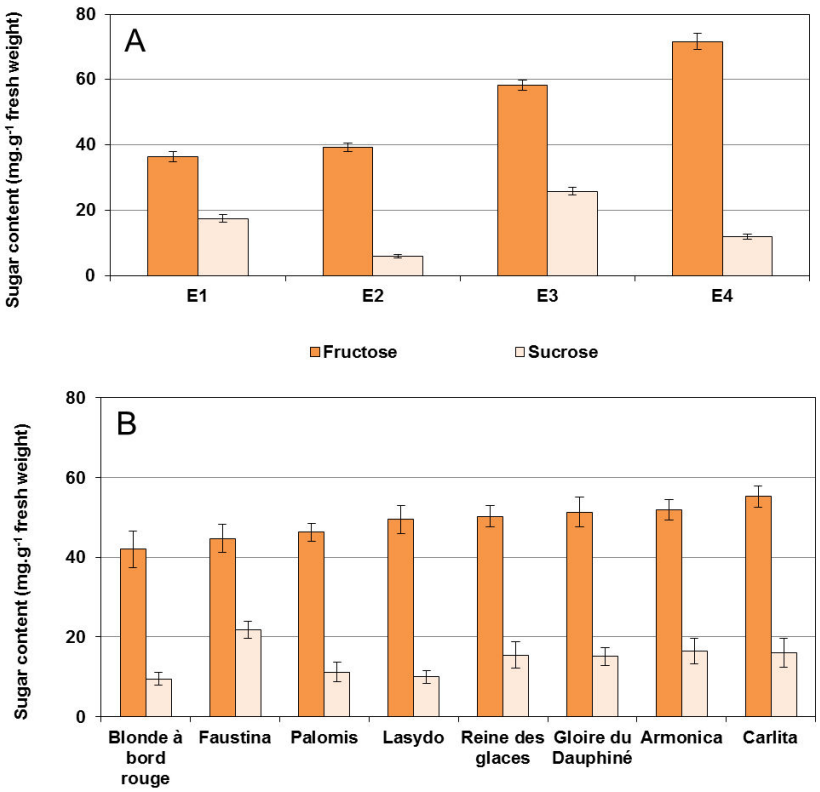

FIGURE 3.3: Variations of fructose and sucrose contents (± standard error of the mean) of lettuce medium-sized leaves in detached leaf experiments (E1 to E4) (means of 5 plants x 9 accessions) (**A**) and accessions (means of 5 plants x 4 experiments) (**B**).

tance levels differing by 5- and 5.5-fold from the most susceptible accession to the most resistant one, with 11 and 20 accessions tested respectively (Whipps et al., 2002 ; Hayes et al., 2010). 'Blonde à bord rouge' was one of the most resistant accessions to *S. sclerotiorum*. Low susceptibility of 'Blonde à bord rouge' (also called Iceberg cultivar or Holborn standard (Rodenburg, 1960)) to *S. sclerotiorum* has already been observed by several authors (Whipps et al., 2002 ; Elia and Piglionica, 1964 ; Hayes et al., 2010), but only at crop level, making it difficult to attribute resistance to plant growth habit or to tissue composition. In the present study, detached leaf experiments suggested that this resistance is independent from plant growth habit. Significant variations in the average rate of lesion expansion among detached leaf experiments were observed for both pathogens. However the classification of accessions with respect to their susceptibility to *B. cinerea* or *S. sclerotiorum* remained generally consistent among experiments, attesting to the stability of the resistance under different environmental conditions (strains and plant growth conditions). This suggests that there is a significant potential for the improvement of resistance to *B. cinerea* and *S. sclerotiorum* in modern cultivars.

Lettuce varieties with low susceptibility to both *B. cinerea* and *S. sclerotiorum* would contribute to reduce the use of pesticides since the two pathogens are usually managed by combined chemical applications in protected lettuce crops (Barrière et al., 2014). We found a positive correlation between the expansion rate of lesions on leaves inoculated with *B. cinerea* and *S. sclerotiorum*. Huang et al. (Huang et al., 2002) also showed a positive correlation between the lesion size of *B. cinerea* and *S. sclerotiorum* (Pearson coef. 0.33) on dry beans. These findings demonstrate that resistance to both pathogens can coexist in the same genotype. However, another necrotrophic pathogen, *Sclerotinia minor* (Jagger), can cause lettuce drop in the field. Although there is no evidence of a positive correlation between resistance to *S. sclerotiorum* and to *S. minor* (Lebeda et al., 2014), reduced susceptibility to *S. minor* has already been observed in several lettuce accessions (Abawi et al., 1980 ; Grube and Ryder, 2004) and accessions with low susceptibility to both *S. sclerotiorum* and *S. minor* have been observed by Hayes et al. (2010).

Except for 'Gloire du Dauphiné', the results of crop level experiment results were in agreement with tests performed on detached leaves, suggesting that a screening method based on detached leaves could be reliable to predict field resistance. 'Gloire du Dauphiné' is a red Batavia type with a voluminous head and large crisp leaves. One or several of its morphological traits may have favored the development of *S. sclerotiorum* in the field. In crop level experiments, disease damage on certain accessions was very low in naturally infested plots even though there was an overall high inoculum pressure within the experimental tunnel. Thus, even in the case of strong pathogen pressure, field resistance can limit the development of *S. sclerotiorum*.

The level of susceptibility to *B. cinerea* and *S. sclerotiorum* in the present study was also influenced by experimental conditions. In earlier work (Lecompte et al., 2013) carried out with a single lettuce accession (cv. Faustina), sucrose and fructose were found to be good indicators of susceptibility to *S. sclerotiorum* (1 strain tested) and *B. cinerea* (6 strains tested including BC1 used in the present study). Here, by assessing several lettuce accessions, we confirmed that sugar content can be correlated to lettuce susceptibility to *B. cinerea* and *S. sclerotiorum*. As in previous work (Lecompte et al., 2013), the susceptibility to *B. cinerea* in this study was positively correlated to fructose content and the susceptibility of *S. sclerotiorum* was negatively correlated to sucrose content.

Soluble sugars are involved in plant-pathogen relationships since they are an important source of carbon for necrotrophic pathogens such as *B. cinerea* and *S. sclerotiorum* (Jobic et al., 2007 ; Dulermo et al., 2009). Furthermore, although their specific action on lettuce susceptibility is unclear, sugars are also likely to be essential for the activation of plant defenses (Berger et al., 2007, Bolton, 2009). They could provide the energy or carbon skeletons required for the establishment of defenses or could be involved in signaling pathways. Knowledge about the action of carbohydrates, including sucrose and hexoses, on plant defense regulation has been recently reviewed (Tauzin and Giardina, 2014). Sugars are clearly involved in the establishment of physical barriers to pathogens (callose deposition), the production of pathogenesis-related proteins and in the regulation of photosynthesis. The deposition of callose is repressed by increased sucrose contents (Tauzin and Giardina, 2014). In our study, since sucrose content was negatively correlated to the rates of lesion expansion, it is unlikely that callose deposition was the underlying resistance

mechanism. Interestingly, we identified sucrose and fructose but not glucose as a key player in plant susceptibility, even though the correlation between glucose and fructose in lettuce leaves was very high. Finding plant primary metabolism indicators could help in ranking the different mechanisms involved in plant defense. Here, we suggest that clear effects on plant susceptibility are governed by fructose and sucrose metabolism, suggesting the activation and repression of defense-based processes and/or virulence-based processes.

The soluble sugar proportions that disadvantage *B. cinerea* development (high sucrose contents and low fructose contents) were consistent with those that disadvantage *S. sclerotiorum* (high sucrose contents, no effect of fructose). Therefore, growing lettuces in conditions that favor such sucrose :fructose proportions would likely reduce both *B. cinerea* and *S. sclerotiorum* development. Plant growing conditions strongly influence carbohydrate contents. Sugar is produced by photosynthesis and is therefore influenced by light intensity, photoperiod and season. The average fructose content observed in our experiments seemed to increase from E1 to E4, *i.e.* with increasing day length. Moreover, in previous work we observed that high light intensity seemed to increase fructose content and decrease sucrose content in lettuce (cv Faustina). Disease development due to *B. cinerea* and *S. sclerotiorum* was favored by high nitrogen fertilization levels, which impacted plant sugar contents (Lecompte et al., 2013). Investigations are needed to better characterize environmental factors affecting sugar content and find how lettuce growing conditions could be manipulated to improve partial resistance to *B. cinerea* and *S. sclerotiorum*. The breeding efforts should therefore focus on the use of accessions showing tissue based- resistance while genotypes and growing conditions should be adapted to achieve low plant fructose :sucrose ratio, unfavorable to *B. cinerea* and *S. sclerotiorum* development.

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Chapitre 4

Effet de la fertilisation azotée et de l'introduction de parasitoïdes sur le développement de *Nasonovia ribisnigri* (Mosley)

Virginie Barrière . Séverin Hatt . Françoise Lescourret . François Lecompte .

Résumé

Nasonovia ribisnigri (Mosley), le puceron de la laitue peut causer d'importantes pertes de rendement dans les cultures de laitues. Son développement, au cœur des laitues le rend à la fois plus préjudiciable que les autres espèces de pucerons attaquant la laitue et moins vulnérable aux insecticides de contact ; si bien que l'utilisation préventive et systématique des insecticides est généralisée en agriculture conventionnelle pour les périodes où la pression de pucerons est la plus forte. L'objectif de cette expérimentation était d'évaluer l'efficacité de techniques alternatives aux insecticides, *i.e.* l'optimisation de fertilisation azotée et l'introduction de parasitoïdes *Aphidius matricariae*, pour gérer les populations de *N. ribisnigri* sur laitue. Des laitues ont été plantées dans 2 tunnels à 2 semaines d'intervalle et soumises à 3 niveaux de fertilisation par tunnel (90, 150 et 210 kgN.ha^{-1} dans le tunnel A et 40, 90, 150 kgN.ha^{-1} dans le tunnel B) répétés 6 fois. Vingt pucerons ailés ont été inoculés par bloc et des parasitoïdes ont été introduits dans une partie de chaque tunnel. Dans les deux tunnels, les pucerons ont affiché une réponse parabolique aux apports d'azotes. Le très faible nombre de momies observées durant l'expérimentation illustre l'incapacité de *A. matricariae* à rencontrer ses hôtes lorsque la laitue est trop développée. Finalement, le mode de dispersion, de proche en proche, des pucerons dans la parcelle, apporte des éléments de réflexion nouveaux pour la gestion de *N. ribisnigri*.

Mots clé

Puceron, laitue, *Aphiduis matricariae*, nitrate, contrôle « top-down » et « bottom-up »

4.1 Introduction

Nasonovia ribisnigri (Mosley)(*Hemiptera*, *Aphididae*) est un puceron ravageur des cultures de laitue (Figure 4.1).

Il présente un cycle holocyclique alternant entre la reproduction sexuée, qui donne naissance à l'œuf d'hiver sur ses hôtes primaires, les plantes du genre *Ribes sp.*, et la reproduction parthénogénétique (Figure 4.2). La laitue cultivée, *Lactuca sativa* L., fait partie des hôtes secondaires de *N. ribisnigri*, tout comme d'autres *Asteraceae* (*Cicorium sp.*, *Crepis sp.*, *Hieracium sp.*, *Lactuca sp.*, *Lampsana sp.*), certaines *Scrophulariaceae* (*Euphrasia sp.*, *Veronica sp.*), et *Solanaceae* (*Nicotiana sp.*, *Petunia sp.*). Le puceron *N. ribisnigri* peut aussi présenter un cycle incomplet (anholocyclique) et se développer toute l'année par parthénogénèse sur la laitue (Nebreda et al., 2005). En hiver, les laitues cultivées sous abris constituent un milieu favorable à la survie des populations puisque le minimum thermique requis pour le développement de *N. ribisnigri* a été estimé à 3-4 ° C (Diaz et al., 2007b). Le taux de survie de *N. ribisnigri* est maximal à 20 ° C (Diaz and Fereres, 2005). A cette température, qui est couramment atteinte sous abri en hiver en zone méditerranéenne, le temps nécessaire au développement d'une génération d'aptères est de 8 jours et celui des ailés est de 9.2 jours.

Les pucerons se nourrissent en plantant leur stylet dans les tissus de la plante hôte et en aspirant la sève élaborée. En plus de provoquer des pertes d'éléments nutritifs pour la plante et d'être vecteurs de virus (Dixon, 1998), les pucerons, par leur simple présence peuvent rendre les laitues non commercialisables (Smith and Chaney, 2007). Par ailleurs, contrairement aux autres espèces de pucerons, *N. ribisnigri* se développe préférentiellement dans le cœur des laitues (Mackenzie and Vernon, 1988). Ce comportement est spécifique à cette espèce de puceron et la rend moins vulnérable aux insecticides de contact (Liu, 2004). Le cœur des laitues étant la partie la plus valorisée de la plante, *N. ribisnigri* peut causer d'importantes pertes de rendement. Pour les cultures conventionnelles de laitues, les seuils de tolérance des Grandes et Moyennes Surfaces (GMS) et des usines de $4^{ème}$ gamme pour la présence d'insectes sont très bas : pas plus de 10% des laitues infestées avec pas plus de 5 individus par plante. Ces seuils ne font, en outre, pas de distinctions entre les ravageurs, comme les pucerons, et les auxiliaires. Pour atteindre ces exigences, les pucerons de la laitue sont contrôlés exclusivement par des applications d'insecticides. Lorsque les conditions climatiques sont propices à leur développement, comme au printemps et en été, des insecticides sont systématiquement appliqués de manière préventive. A l'automne et en hiver, les insecticides peuvent être moins systématiques et déclenchés lorsque les pucerons sont observés dans la zone de production. Au sein des populations de *N. ribisnigri*, des résistances à différentes familles d'insecticides telles que les pirimicarbes, les pyréthrinoïdes et les organophosphates ont été observées (Barber et al., 1999, Kift et al., 2004, Rufingier et al.,

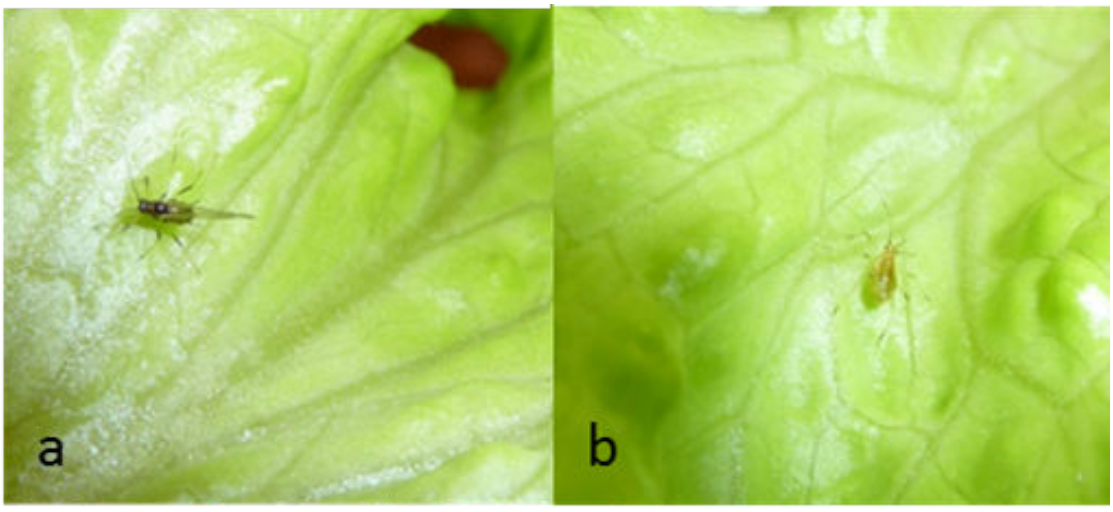

FIGURE 4.1: Individu adulte ailé (**a**) et aptère (**b**) de *N. ribisnigri*. Les ailés sont verts pâles à verts foncés et ont des antennes longues et noires. Les aptères sont jaunes à verdâtres et possèdent sur l'arrière de l'abdomen des tâches sombres et étroites qui sont disposées de chaque côté de la ligne médiane.

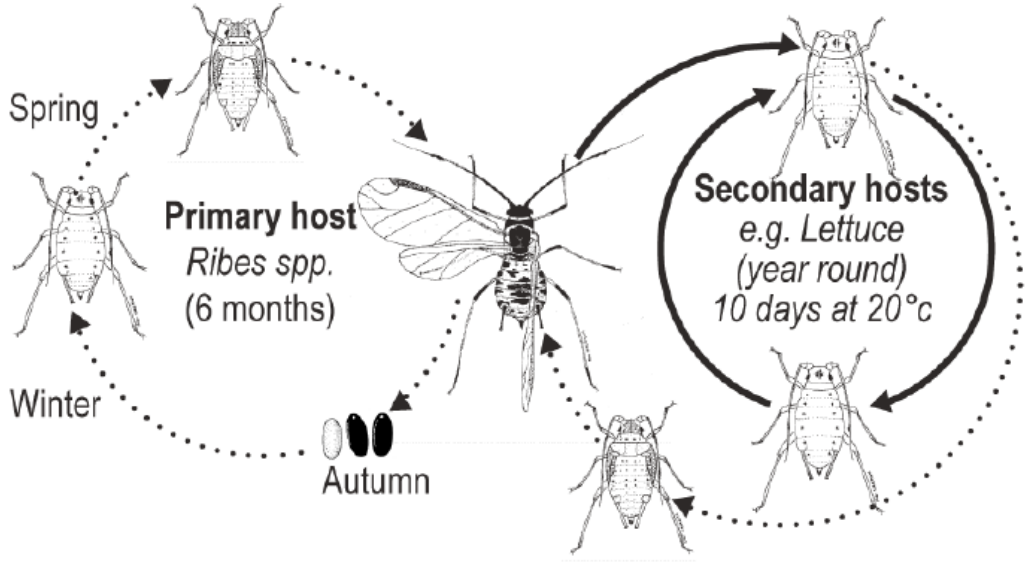

FIGURE 4.2: Cycle biologique de *Nasonovia ribisnigri* (d'après McDougall and Creek, 2011).

1999).

En agriculture biologique, les seuils de tolérance pour la présence d'insectes sur les laitues sont plus élevés qu'en agriculture conventionnelle. Cependant, en l'absence d'alternative égalant l'efficacité des insecticides de synthèse, les rendements dépendent fortement des pressions de pucerons.

Plusieurs techniques alternatives aux insecticides ont été étudiées pour gérer les populations de pucerons sur différentes cultures. Néanmoins, leur efficacité, seulement partielle, leur coût, parfois élevé et le manque d'études réalisées spécifiquement sur laitue pourraient être des freins importants pour l'intégration de ces techniques dans les stratégies de gestion des pucerons. Ces techniques peuvent être classées dans 3 catégories : i) les techniques physiques limitant les interactions entre plante et pucerons, ii) les techniques ayant un effet « bottom-up », c'est-à-dire via les ressources offertes aux pucerons et iii) les techniques ayant un effet « top-down », c'est-à-dire via les niveaux trophiques supérieurs, sur les populations de pucerons.

Les techniques physiques de gestion des pucerons limitent les interactions entre la plante et les pucerons. Par exemple, l'installation de filets insect-proof peut fournir une protection efficace sous abris mais aussi en plein champs (Weintraub and Berlinger, 2004). Néanmoins, ces structures présentent l'inconvénient d'augmenter les températures et, sous certaines conditions, l'humidité en limitant les flux d'air, ce qui peut créer des conditions potentiellement plus favorables au développement d'agents pathogènes. Les filets sont aujourd'hui peu utilisés en production car leur coût assez élevé et leur impact sur les conditions climatiques limitent leur adoption par les producteurs. D'autres techniques physiques de gestion des pucerons reposent sur le fait que la lumière joue un rôle important dans l'établissement des interactions plantes- pucerons (Raviv et Antignus, 2004). Des modifications du spectre lumineux peuvent altérer certains comportements des insectes ravageurs comme l'orientation, la recherche de nourriture et d'hôtes. Ainsi, la couleur du paillage ou l'interception de certaines longueurs d'ondes par les films de protection des tunnels peuvent influencer les interactions plantes-pucerons. Fricke et Piepenbrock (2005) ont observé cinq fois moins de pucerons ailés sur des laitues plantées sur un paillage de couleur argent que sur un paillage de couleur vert. Diaz et al. (2006) ont montré que l'utilisation de film anti-ultraviolet pouvait réduire l'abondance des pucerons (*Macrosiphum euphorbiae*), des thrips (*Frankliniella occidentalis*) et des noctuelles (*Autographa gamma*) sur laitue. Les techniques agissant sur la lumière ne sont pas utilisées en condition de production, probablement car elles agissent également sur des facteurs pouvant influencer la qualité des laitues (production d'anthocyanes, température du sol).

Les techniques ayant un effet « bottom-up » visent à contrôler les populations de pucerons via la plante hôte. Jusqu'à récemment, des variétés de laitues résistantes à *N. ribisnigri* étaient utilisées. Cette résistance reposant sur un gène majeur, les fortes pressions de sélection exercées sur les populations de *N. ribisnigri* (souche Nr :0) ont conduit à son contournement par une nouvelle souche nommée Nr :1(van der Arend, 2003). La résistance à Nr :0 apparait encore sur les catalogues des semenciers mais le choix variétal des producteurs en agriculture conventionnelle n'est plus orienté par ce caractère. Des programmes de sélection de variétés résistantes à Nr : 1 sont en cours (Cid et al., 2012). La manipulation de l'état physiologique des plantes pourrait également permettre d'exercer des forces « bottom-up » sur les pucerons. La nutrition azotée de la plante, qui joue un rôle majeur dans la synthèse des acides aminées et des protéines, exerce des effets "bottom-up" sur les niveaux trophiques supérieurs (Chen et al., 2010) en influençant la qualité de la ressource nutritive pour les herbivores. La qualité de la sève élaborée, notamment la proportion d'acides aminés essentiels, dépend de la disponibilité en azote dans le sol (Ponder et al., 2000). De plus, les capacités de la plante à exprimer des défenses constitutives ou induites face aux herbivores ou à libérer des composés volatils sont influencées par la nutrition des plantes (Chen and Ruberson, 2008 ; Chen et al., 2010 ; Veromann et al., 2013). Bien que les résultats des études évaluant l'effet des fertilisants sur les insectes herbivores soient parfois contradictoires, la littérature va généralement dans le sens d'une corrélation positive entre fertilisation azotée et croissance des populations de pucerons (Butler et al., 2012). A notre connaissance, aucune étude n'a été menée à ce jour sur l'effet de la fertilisation azotée des laitues sur *N. ribisnigri*.

Finalement, la lutte biologique peut avoir des effets « top-down » sur les populations de pucerons. De nombreuses espèces de prédateurs polyphages peuvent participer à la gestion des populations de pucerons en culture de laitues. Plusieurs espèces de *Syrphidae*, de *Coccinellidae* et de *Chrysopidae* sont prédatrices des pucerons de la laitue (Farsi et al., 2014). Les larves de *Chrysoperla carnea*, par exemple, peuvent ingérer jusqu'à 270 pucerons de l'espèce *Myzus persicae* par jour (Liu et Chen, 2001). Cependant, l'introduction de prédateurs n'est pas en adéquation avec les exigences des acheteurs de laitue en termes de qualité visuelle et d'absence de corps étrangers. Les prédateurs sont en effet au même titre que les pucerons considérés comme des corps étrangers.

Contrairement aux prédateurs, les parasitoïdes ne se nourrissent pas des pucerons mais s'en servent pour effectuer une partie de leur cycle de vie. Les femelles adultes pondent à l'intérieur des pucerons. Les œufs éclosent et les larves se développent en se nourrissant des entrailles du puceron. Le puceron meurt alors en se momifiant et le nouvel adulte parasitoïde émerge en créant un opercule sur la momie. La présence de momies de pucerons en petite quantité est davantage tolérée que celle des prédateurs par les acheteurs de laitue. Parmi les hyménoptères parasitoïdes, plusieurs espèces de genre *Aphidius*, telles que *A. colemani*, *A. hieraciorum* and *A. matricaria*, ont été identifiées comme étant des parasitoïdes de *N. ribisnigri* (Nebreda et al., 2005, Kavallieratos et al., 2004, Farsi et al., 2014). Leur capacité à parasiter les pucerons dépend en partie de la complexité de la structure du couvert végétal qui peut influencer la capacité des parasitoïdes à rencontrer leurs hôtes (Andow and Prokrym, 1990). Or, l'architecture des laitues en fin de culture est relativement complexe et le cœur des laitues, dans lequel se développe *N. ribisnigri*, est un milieu protégé, surtout si la laitue tend à pommer. La capacité des *Aphidius* spp. à parasiter *N. ribisnigri* en conditions de production doit être examinée. La plante, par ses effets « bottom-up » sur les populations de pucerons, peut également avoir d'autres effets indirects sur les niveaux trophiques supérieurs (Aqueel et al., 2014). D'une part, l'efficacité du parasitisme dépend de la densité de pucerons (Zamani et al., 2006). D'autre part, les parasitoïdes se nourrissent du miellat des pucerons dont la valeur nutritive pourrait être directement liée à l'alimentation des pucerons et donc à la plante hôte (Douglas, 2006, Hogervorst et al., 2007). L'utilisation des techniques à effet « top-down » requiert donc de s'intéresser également aux techniques ayant un effet « bottom-up » sur les populations de pucerons.

Les objectifs de cette étude ont été de déterminer i) comment la fertilisation azotée et l'introduction de parasitoïdes pouvaient impacter le développement de *N. ribisnigri*. Et également ii) de vérifier la compatibilité de ces techniques pour une utilisation potentielle en combinaison pour gérer les populations de pucerons sur laitue.

4.2 Matériel et Méthodes

4.2.1 Dispositif expérimental et matériel végétal

L'expérimentation a été réalisée entre avril et juin 2013 au domaine St Paul de l'INRA d'Avignon (43 ° 91'N ; 4 ° 87'E ; altitude : 31m). Des laitues de la variété Astrapi (Gautier semences) ont été plantées à la densité de 14.25 plantes par m^2 dans deux tunnels de 210 m^2 munis aux portes et ouvrants de filets insect-proof de maille 510 x 510 μm. Les tunnels étaient également séparés en deux à l'aide d'un filet insect-proof de la même maille.

Les dates de semis, de plantation, d'inoculation et de récolte des laitues ont été décalées de 2 semaines environ entre les deux tunnels afin d'appréhender l'effet des conditions climatiques et d'ajuster le protocole si nécessaire.

Tunnel A :
Date de semis : 06/03/2013
Date de plantation : 26/03/2013
Date d'inoculation : 25/04/2013
Date de récolte prévue : 16/05/2013

Tunnel B :
Date de semis : 18/03/2013
Date de plantation : 09/04/2013
Date d'inoculation : 09/05/2013
Date de récolte prévue : 30/05/2013

4.2.2 Niveaux de fertilisation

Trois niveaux de fertilisation ont été testés dans chaque tunnel. Initialement, nous avions prévu d'appliquer les mêmes traitements azotés dans les deux tunnels : un niveau faible (40 kgN.ha^{-1}), un niveau intermédiaire (90 kgN.ha^{-1}) et un niveau élevé (150 kgN.ha^{-1}). Cependant, dans le tunnel A le reliquat azoté du sol était supérieur à 40 kgN.ha^{-1}. Pour ce tunnel les niveaux suivants ont donc été utilisés : 90, 150 et 210 kgN.ha^{-1} (Figure 4.3). Le dispositif était constitué de blocs randomisés avec trois répétitions par demi-tunnel. Pour chaque tunnel, un demi-tunnel était consacré à l'étude de la dynamique des pucerons seuls, et l'autre demi-tunnel à la dynamique des pucerons avec introduction de parasitoïdes (voir plus loin). Les blocs ont été répartis aléatoirement au sein des demi-tunnels, avec une symétrie entre les deux demi-tunnels, à l'exception de deux blocs dans le tunnel B côté sud qui ont été déplacés car les teneurs en azote du sol pour ces blocs étaient supérieures aux niveaux de fertilisation prévus. Pour chaque bloc, des échantillons de sol ont été prélevés une semaine avant inoculation sur 30 cm de profondeur et la teneur en nitrate du sol a été mesurée. Afin d'ajuster la teneur en nitrate du sol au niveau de fertilisation désiré, de l'ammonitrate a été apporté directement aux pieds des laitues et une courte irrigation a été réalisée trois jours avant l'inoculation.

4.2.3 Elevage des pucerons

L'élevage des pucerons a été réalisé à partir d'une souche de *N. ribisnigri* fournie par l'unité Santé de la Vigne et Qualité du Vin (SVQV) de l'INRA de Colmar. Les pucerons *N. ribisnigri* ont été élevés sur des laitues de la variété Astrapi avec une température constante de 22 ° C et une photopériode de 16 :8 h afin de maximiser la proportion d'ailés. Dans un premier temps, 180 pucerons adultes ont été déposés sur 15 laitues puis retirés 24 h après. Les individus larviposés pendant ce laps de temps se sont développés sur ces mêmes plantes. Au bout de sept jours, les adultes nouvellement formés ont été prélevés et déposés sur 15 nouvelles laitues (cv. Astrapi) et retirés 24 h plus tard. Trois cycles ont été nécessaires pour obtenir une population synchrone d'au moins 540 ailés nécessaires à chaque inoculation. L'inoculum, protégé par une cage insect-proof, a été transféré dans le tunnel 24 h avant l'inoculation afin que les pucerons s'adaptent aux fluctuations climatiques quotidiennes.

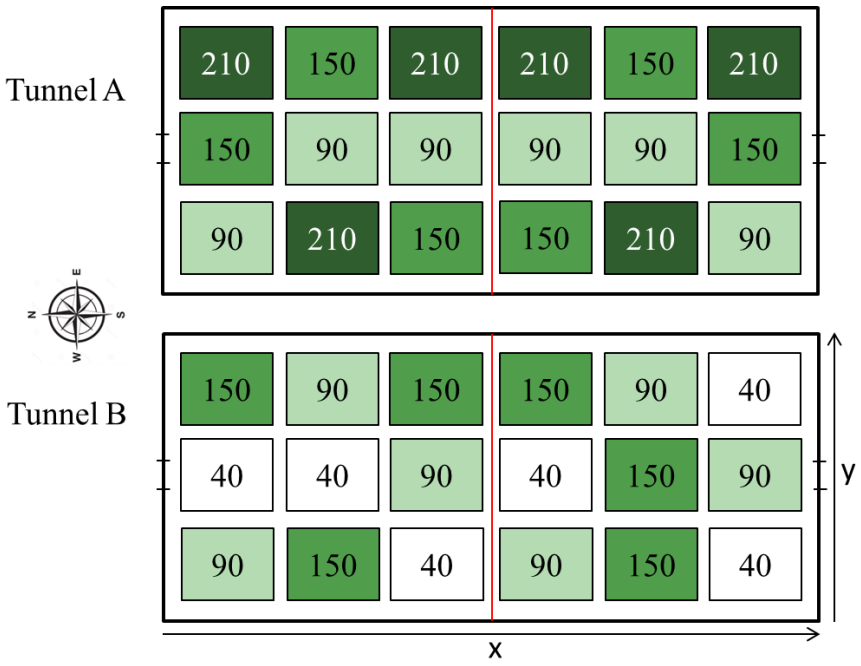

FIGURE 4.3: Teneurs en nitrate du sol (kg.ha^{-1}), ajustées 3 jours avant inoculation des pucerons. Chaque niveau de fertilisation est répété trois fois par demi-tunnel. La ligne rouge représente le filet insect-proof central séparant les deux côtés du tunnel. Des parasitoïdes (*A. matricariae*) de *N. ribisnigri* ont été introduits côté sud des deux tunnels d'expérimentation

4.2.4 Inoculation et comptage

Cinq semaines après la plantation, quatre laitues par bloc, situées au centre du bloc, ont été inoculées chacune avec cinq individus ailés dans chaque demi-tunnel. Les pucerons ont été déposés au cœur des laitues. Ainsi, chaque bloc a été inoculé avec 20 pucerons ailés, soit 180 individus par demi-tunnel.

Le nombre total de pucerons (larves et adultes) sur les plantes inoculées a été compté tous les trois à quatre jours pendant trois semaines suivant l'inoculation. Les pucerons ont également été dénombrés sur huit autres plantes de chaque bloc, dont la position était identique dans les blocs et dont la distance avec le point d'inoculation était de 0.37 m (distance notée d1) pour 4 d'entre elles et de 1.11 m (distance notée d2) pour les 4 autres.

4.2.5 Introduction des parasitoïdes *Aphidius matricariae*

A partir de l'inoculation, des parasitoïdes *Aphidius matricariae* (Aphipar-M®, Koppert) ont été introduits, une fois par semaine, sous forme de momies à la dose de un *A. matricariae* pour dix pucerons inoculés dans le côté sud du tunnel A et à raison de cinq *A. matricariae* pour dix pucerons inoculés dans le côté sud du tunnel B. Les momies ont été comptées sur chaque plante observée lors du comptage des pucerons.

4.2.6 Récolte

Dans chaque tunnel, trois semaines après inoculation, les plantes étaient prêtes à être récoltées. Les laitues observées tout au long de l'expérimentation, y compris les plantes inoculées, ont été prélevées et ensachées individuellement. Chacune a ensuite été placée sur une feuille de papier blanc A3 et un comptage destructif des pucerons et des momies a été réalisé.

Par ailleurs, six laitues par bloc, sélectionnées aléatoirement, ont été pesées. Puis trois feuilles de la couronne intermédiaire de trois laitues sélectionnées aléatoirement par bloc (3 feuilles x 3

laitues) ont été prélevées, immédiatement placées dans l'azote liquide et conservées à -20 ° C. Des sous échantillons de matière sèche ont été calcinés à 400 ° C pendant 12 h puis minéralisés dans de l'acide nitrique (HNO_3) bouillant. La teneur en nitrate a été mesurée avec un analyseur NO_3 / NO_2 (FIAstar 5000).

4.2.7 Analyse des données

Les analyses statistiques ont été effectuées à l'aide du logiciel R 3.1.1 (R-Core-Team, 2013). Du fait des différences de date d'inoculation, de niveau de fertilisation et de densité de parasitoïdes, les données obtenues dans les deux tunnels ont été analysées séparément.

Effet de la fertilisation azotée sur la teneur en nitrate des feuilles

L'effet de la fertilisation azotée sur la teneur en nitrate des feuilles a été testé en utilisant une analyse de variance (ANOVA). Les trois traitements de chaque tunnel ont été comparés en utilisant un test de comparaison des moyennes de Student Newman Keuls. Considérant la variabilité des teneurs en nitrate exprimées en réponse à la fertilisation azotée, pour le reste de l'analyse, quatre classes de teneur en nitrate des feuilles ont été déterminées en utilisant les quartiles des données obtenues pour les deux tunnels. Les différents niveaux de teneur en nitrate des feuilles sont notés :
N1 : valeurs < 240 μmol $NO_3.g^{-1}$ matière sèche,
N2 : valeurs comprises entre 240 et 280 μmol $NO_3.g^{-1}$ matière sèche,
N3 : valeurs comprises entre 280 et 330 μmol $NO_3.g^{-1}$ matière sèche,
N4 : valeurs > 330 μmol $NO_3.g^{-1}$ matière sèche.

Effet de la fertilisation sur le poids des laitues

Deux ANOVA ont été réalisées avec le poids des laitues comme variable à expliquer. L'une a été effectuée avec les coordonnées des blocs (x et y) et le niveau de fertilisation en variables explicatives, l'autre avec les coordonnées des blocs (x et y -cf. Fig. 4.3) et le niveau de nitrate dans les feuilles en variables explicatives.

Effet de la teneur en nitrate des feuilles sur le nombre de pucerons par plante à la récolte

Pour chaque tunnel, un modèle linéaire généralisé a été construit avec le nombre de pucerons moyen par plante à la récolte comme variable à expliquer, les coordonnées du bloc (x et y) dans le tunnel et le niveau de nitrate des feuilles comme variables explicatives. Un test de Student Newman Keuls a ensuite été effectué pour comparer le nombre moyen de pucerons en fonction des niveaux NO_3 et des coordonnées x et y.

Analyse de la dispersion des pucerons

Les taux d'accroissement de la population de pucerons pour chaque bloc ont été calculés entre la première observation et la troisième observation (R1-3), puis entre le troisième et la cinquième observation (R3-5) de la manière suivante :

$$Ri - j = (Pj - Pi)/Pi$$

Avec Pi : la somme (+1, de manière à ne pas avoir de dénominateur nul) des pucerons pour les 4 plantes (point d'inoculation, d1 ou d2) observées à la date initiale i et Pj : la somme (+1) des pucerons pour les 4 plantes (point d'inoculation, d1 ou d2) observées à la date ultérieure j. Pour chaque tunnel, une ANOVA a été réalisée avec le taux d'accroissement (R1-3 ou R3-5) transformé en log (x+1) comme variable à expliquer et la distance au point d'inoculation comme variable explicative. Lorsque la distance présentait un effet significatif sur le taux d'accroissement, un test de Student Newman Keuls a été utilisé pour comparer les taux d'accroissement entre distances. Finalement, une interpolation du nombre de pucerons par plante pour chaque distance

a été réalisée et le nombre théorique total de pucerons par bloc a été calculé pour chaque date d'observation.

4.3 Résultats

4.3.1 Effet de la fertilisation sur la teneur en nitrate des feuilles de laitue

Pour tous les niveaux de fertilisation azotée appliqués, les teneurs en nitrate observées étaient largement en dessous des normes en vigueur (seuil légal UE : 3500 ppm matière fraiche soit environ 1130 μmol.g^{-1} matière sèche). La fertilisation azotée a influencé la teneur en nitrate des feuilles de laitues à la récolte dans le tunnel A (F= 7.1095 ; 2df ; P= 0.006733) et dans le tunnel B (F= 4.1357 ; 2df ;P= 0.03711). Dans le tunnel A, seule la teneur en nitrate des feuilles de laitues cultivées avec un niveau fertilisation azotée de 90 kg.ha^{-1} était significativement différente des autres traitements. Dans le tunnel B, les teneurs en nitrate dans les laitues étaient significativement différentes entre les niveaux 40 et 150 kg.ha^{-1} (Tableau 4.1).

Niveaux de fertilisation azotée (kgN.ha^{-1})	Teneur en nitrate des feuilles (μmol.g^{-1} matière sèche)	
	Tunnel A	Tunnel B
40	-	201.4 (± 69.3) a
90	280.3 (± 47.7) a	249.6 (± 81.6) ab
150	324.5 (± 56.2) b	287.0 (± 82.1) b
210	355.0 (± 58.1) b	-

TABLE 4.1: Teneurs en nitrate de laitues (moyennes de 18 échantillons) cultivées dans les tunnels A et B sous différents niveaux de fertilisation azotée. Les lettres donnent le résultat du test de comparaison multiple des moyennes (Student Newman Keuls).

Bien que les teneurs en nitrate moyennes soient cohérentes avec les niveaux de fertilisation azotée appliqués, il y a une importante variabilité de réponses pour un même niveau de fertilisation. Pour chaque niveau de fertilisation, 2 à 3 niveaux de teneurs en nitrate des feuilles ont été observés (Figure 4.4). L'effet de la fertilisation azotée sur les pucerons et le parasitisme a donc été étudiée en utilisant les niveaux de teneur en nitrate qui étaient plus représentatifs de la nutrition azotée des laitues que les niveaux de fertilisation appliqués.

4.3.2 Effet de la teneur en nitrate sur le poids des laitues

Le poids moyen des laitues était de 468g (±51) dans le tunnel A et de 579g (±67) dans le tunnel B. Dans le tunnel A comme dans le tunnel B, le poids des laitues à la récolte n'a été affecté ni par les niveaux de fertilisation (Tunnel A : F=1.491, 2 df, P= 0.25312 ; Tunnel B : F= 0.007, 2df, P=0.934485) ni par la teneur en nitrate des feuilles (Tunnel A : F= 1.156, 2df, P= 0.36209 ; Tunnel B : F= 0.054, 2df, P= 0.948214). Les coordonnées du bloc x et y, en revanche, ont eu un impact sur le poids des laitues dans le tunnel A (x : F= 7.176, 5df, P=0.00787 ; y : F= 12.636, 2df, P= 0.00334) et dans le tunnel B (x : F= 14.525, 5df, P=0.000773 ; y : F= 7.801, 2df, P= 0.013199).

4.3.3 Effet de la teneur en nitrate sur la densité de pucerons

Dans le tunnel A, la densité de pucerons par plante a été significativement affectée par la position du bloc dans la longueur du tunnel (x) (F=7.5887 ; 5df ; P <0.0001) et par le niveau de nitrate dans les feuilles (F= 4.7348 ; 2df ; P= 0.009787). La plus forte densité a été observée sur les plantes dont la teneur en nitrate était comprise entre 240 et 280 μmol.g^{-1} MS (niveau N2)

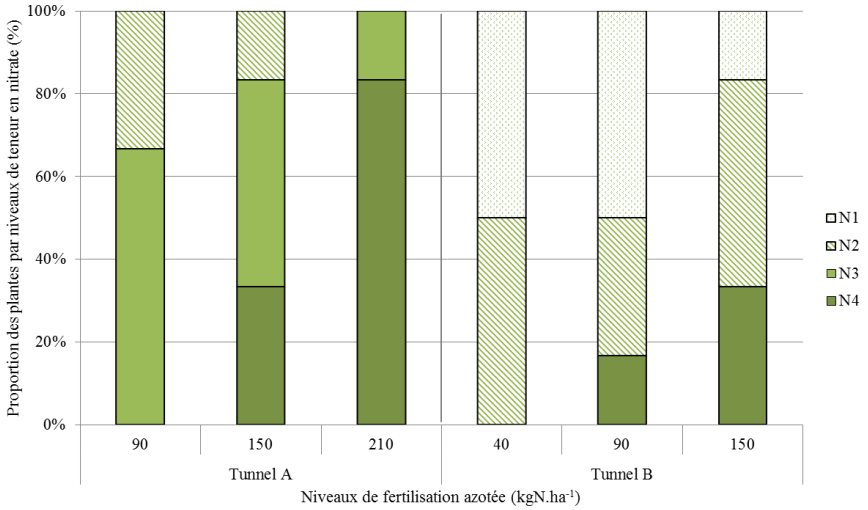

FIGURE 4.4: Proportion des plantes présentant différents niveaux de teneur en nitrate pour chaque niveau de fertilisation azotée dans les tunnels A et B

alors que la plus faible densité a été observée lorsque les plantes contenaient plus de 330 μmol.g^{-1} MS (niveau N4) (Figure 4.5). Dans le tunnel B, la densité de pucerons par plante a aussi été significativement affectée par la position du bloc dans la longueur du tunnel (x) (F= 15.2931 ; 5df ; P <0.0001) et par le niveau de nitrate dans les feuilles (F= 3.1112 ; 2df ; P= 0.04685). Cette fois, la plus forte densité de pucerons a été observée avec la teneur en nitrate N3 (entre 280 et 330 μmol.g^{-1} MS) et la plus faible avec la teneur N2 (entre 240 et 280 μmol.g^{-1} MS) (Figure 4.5). Dans les deux tunnels, les pucerons ont affiché une réponse parabolique aux apports d'azotes. La densité maximale de pucerons n'a pas été atteinte pour le même niveau de nitrate des feuilles ce qui suggère que l'effet du nitrate s'est exprimé en interaction avec les conditions expérimentales. Le dispositif expérimental ne nous a cependant pas permis de tester cette interaction.

4.3.4 Dispersion spatiale de *N. ribisnigri*

Les taux d'accroissement de la population de pucerons pour chaque distance entre la première et la troisième observation (R1-3) ne sont pas significativement différents dans le tunnel A (F= 0.863 ; 1 df ; P= 0.357). Par contre, le taux d'accroissement R3-5 au point d'inoculation dans le tunnel A est significativement différent des taux d'accroissement calculés en d1 et en d2 (F= 15.01 ; 1df ; P= 0.000302). Dans le tunnel B, R1-3 et R3-5 au point d'inoculation sont significativement différents de R1-3 (F= 4.429 ; 1df ; P= 0.0402) et R3-5 (F= 4.584 ; 1df ; P= 0.037) aux distances d1 et d2.

Dans le tunnel A, le nombre total de pucerons au point d'inoculation a chuté fortement après la troisième observation et a ensuite ré- augmenté faiblement (Figure 4.6). Dès leur apparition sur les laitues situées en d1 et d2, et lors des observations suivantes et à la récolte (données non montrées), les pucerons étaient plus nombreux à d1 qu'à d2. Dans le tunnel B, le nombre total de pucerons est resté constant au point d'inoculation tout au long de l'expérimentation. Comme dans le tunnel A, les pucerons étaient plus nombreux à d1 qu'à d2 (excepté pour l'observation 2) y compris à la récolte (données non montrées). Des pucerons ailés ont été observés aux distances d1 et d2 lors de la première observation qui a eu lieu trois jours après inoculation. Les ailés ont donc activement participé à la migration de la population depuis le point d'inoculation.

La dispersion des pucerons au sein des blocs s'est opérée de proche en proche à partir du point d'inoculation. La dispersion au sein de chaque demi-tunnel est présentée dans la Figure 4.7. Le niveau d'infestation est beaucoup plus important dans le tunnel B que dans le tunnel A. Seulement certains points d'inoculation constituent des foyers d'infestation. Dans les deux tunnels, les pucerons s'étendent dans toutes les directions à partir de plusieurs foyers d'infestation.

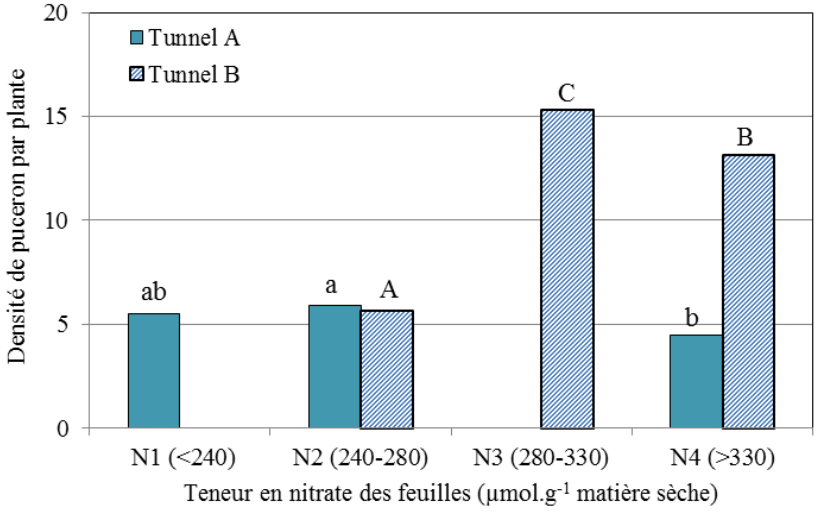

FIGURE 4.5: Effet de la teneur en nitrate des feuilles de laitues à la récolte sur la densité moyenne de pucerons dans le tunnel A et le tunnel B

4.3.5 Parasitisme de *N. ribisnigri* par *A. matricarie*

Aucune momie n'a été observée dans le tunnel A. Dans le tunnel B, seulement 22 momies ont été observées, majoritairement des momies d'aptères mais aussi des momies de pucerons ailés. Leur nombre n'était pas suffisant pour permettre une analyse statistique. Cependant, il est intéressant de constater que les momies ont été exclusivement observées sur des plantes pour lesquelles la densité moyenne de pucerons était supérieure à 10 et dont la teneur en nitrate était supérieure à 280 μmol $NO_3.g^{-1}$ matière sèche (niveaux N3 et N4).

4.4 Discussion

Dans cette étude, une influence de la teneur en nitrate des feuilles de laitues, et par extension de la fertilisation azotée, sur la densité de pucerons *N. ribisnigri* a été mise en évidence. Les plantes présentant des niveaux intermédiaires N2 et N3 de teneur en nitrate des feuilles, qui correspondent aux niveaux de fertilisation azotée couramment appliqués sur les cultures de laitues, ont été les plus infestées par les pucerons. La population de *N. ribisnigri* n'a, ainsi, pas suivi la tendance générale de corrélation positive entre densité de pucerons et apports d'azote décrite par Butler et al. (2012). Ces résultats sont, cependant, à nuancer car des différences de densité de pucerons et de poids de laitues imputables à la position des blocs au sein des tunnels d'expérimentations ont été observées. Néanmoins, les résultats de cette étude sont en accord avec ceux d'autres auteurs qui ont montré que la réponse des populations de pucerons à une augmentation de la disponibilité en azote était parabolique avec une densité de pucerons réduite pour les faibles et les hauts niveaux de fertilisation azotée (Sauge et al. 2012 ; Gash et al. 2012).

Dans la présente étude, certains points d'inoculation ont constitué d'importants foyers d'infestation, tandis que d'autres n'ont pas permis le développement d'une population de pucerons à partir des individus inoculés. Les pucerons se sont dispersés de proche en proche, à partir de quelques points d'inoculation, conduisant à une répartition hétérogène des individus dans les tunnels. Un mode de dispersion similaire a été mis en évidence par Diaz et al. (2012) sur des laitues romaines cultivées sous serre. Les pucerons ont colonisé les plantes adjacentes plutôt que les plantes éloignées du point d'inoculation. Il est probable que le passage d'une laitue à l'autre se soit effectué directement en marchant de feuille à feuille puisque les laitues se touchaient dès l'inoculation. Puisqu'ils ont été observés à la distance d2 dès le troisième jour suivant l'inoculation, les ailés ont, tout comme les aptères, participé, en marchant, à cette colonisation de proche

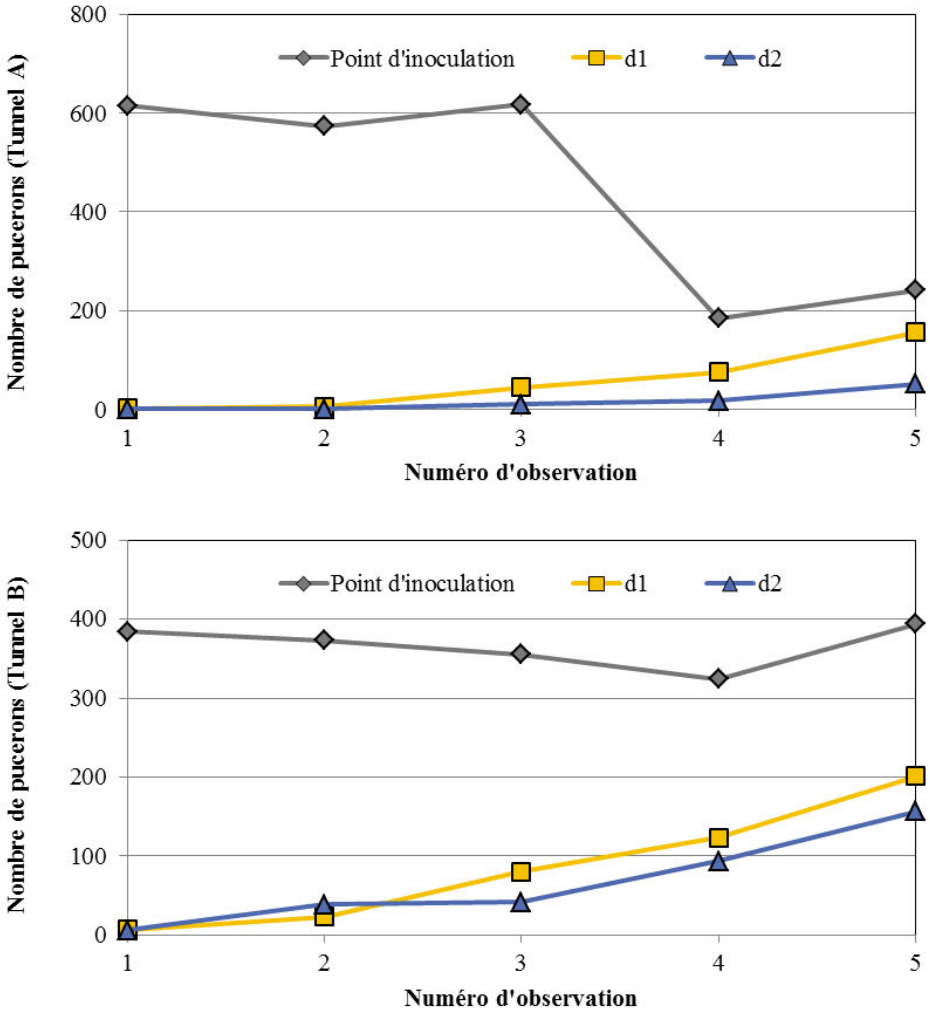

FIGURE 4.6: Evolution du nombre total de pucerons (somme de 24 échantillons) en fonction du nombre d'observations réalisées depuis l'inoculation et de la distance au point d'inoculation dans le tunnel A et le tunnel B.

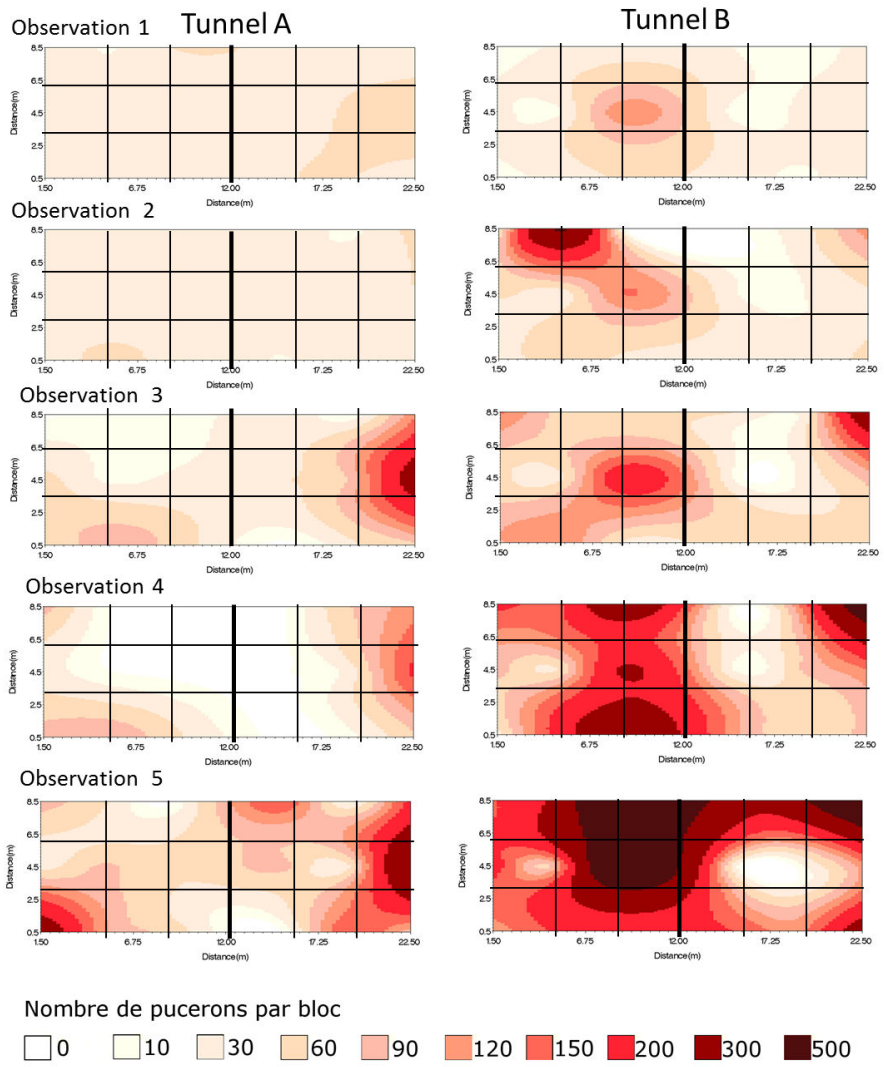

FIGURE 4.7: Dispersion spatiale des pucerons dans le tunnel A et le tunnel B tout au long de l'expérimentation. Graphique obtenu par interpolation des densités de pucerons par bloc.

en proche. Ces résultats suggèrent que le vol des ailés pourrait être réservé à la migration à plus grande échelle que celle de la parcelle. Cette hypothèse est appuyée par l'étude de Liu (2004) qui a détecté des capacités de vol limitées chez les ailés de l'espèce *N. ribisnigri*. Diaz et al. (2012) sont arrivés à des conclusions similaires à ceci près que les plantes ne se touchaient pas et que les pucerons ont, en conséquence, colonisé les plantes adjacentes à la plante inoculée en marchant sur le sol nu. Puisque la présente étude et celle de Diaz et al. (2012) divergent en de nombreux points du protocole (densité de plantation, inoculation, températures, etc.), il est difficile de comparer les distances parcourues par les pucerons. Néanmoins, il est probable que la colonisation des plantes adjacentes soit plus efficace lorsque les plantes se touchent. Dans ce cas, il y a une continuité de structure (feuille-feuille) alors que lorsque les plantes sont espacées, même de quelques centimètres, le puceron doit à la fois marcher sur une nouvelle structure (sol ou paillage) et repérer son prochain hôte.

Le très faible nombre de momies observé dans cette étude montre que les parasitoïdes introduits côté sud de chaque tunnel n'ont pas impacté les populations de *N. ribisnigri*. La complexité du couvert végétal au moment de l'introduction des parasitoïdes (3 semaines avant la récolte) et la préférence de *N. ribisnigri* pour se développer au cœur des laitues ont pu constituer des freins importants à la rencontre des hôtes par les parasitoïdes. Les momies de pucerons ont été observées uniquement sur les laitues qui présentaient des densités de pucerons importantes et des teneurs en nitrate élevées (N3 et N4). Généralement pourtant, les plantes déficientes en azote produisent davantage de VOC susceptibles d'attirer les parasitoïdes (Chen and Ruberson, 2008). Chen et al. (2010) ont suggéré que l'augmentation du parasitisme sur des plantes ayant reçu de hauts niveaux de fertilisation azoté, observée dans plusieurs études (Fox et al., 1990 ; Bentz et al. 1996) pourrait résulter d'une augmentation de la fitness des parasitoïdes se développant sur ces plantes et non d'une augmentation du recrutement des parasitoïdes. La culture de laitue ne présentant pas d'intérêt pour la nutrition des parasitoïdes (absence de fleur, de nectar), nous suggérons que le nombre accru de momie sur les plantes N3 et N4 dans la présente étude résulte d'un effet indirect de la fertilisation azotée via l'augmentation des densités de puceron qui sont ainsi plus facilement repérables par les parasitoïdes (Zamani et al., 2006).

4.5 Conclusion

Les niveaux de fertilisation azotée couramment utilisés pour les cultures de laitues conventionnelles (90 à 150 kgN.ha^{-1}) semblent favoriser le développement de *N. ribisnigri*. Une diminution de la fertilisation pourrait permettre de réduire les infestations, néanmoins, au regard de certaines faiblesses du dispositif expérimental, d'autres tests sont nécessaires pour appuyer ces conclusions. Les introductions de parasitoïdes *A. matricariae*, 3 semaines avant la récolte, n'ont pas impacté les populations de pucerons et ne devraient pas être préconisées dès lors que la complexité du couvert végétal devient trop importante. Le schéma de dispersion de *N. ribisnigri* observé dans la présente étude à l'échelle de la parcelle que la surveillance des pucerons doit être effectuée à plusieurs endroits dans la parcelle pour évaluer efficacement la pression de ravageurs. Le mode de dispersion de proche en proche devrait être mieux caractérisé, notamment le passage des pucerons d'une plante à l'autre lorsque les plantes sont en contact ou espacées. Certaines pratiques culturales, telle que la géométrie de plantation, pourraient être repensées en conséquence, et de nouvelles techniques de gestion des pucerons, telles de l'utilisation d'un paillage ralentissant la dispersion, pourraient être développées sur la base de ce mode de dispersion.

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Troisième partie

APPROCHE INTÉGRATIVE : Efficacité et performances économiques et environnementales des stratégies alternatives de gestions des bio-agresseurs de la laitue

Chapitre 5

Efficacy of pest and pathogen control, yield and quality of winter lettuce crops managed with reduced pesticide applications [1]

Virginie Barriere . François Lecompte . Françoise Lescourret

Abstract

In conventional agriculture, lettuce crops receive large amounts of pesticides to meet stringent industrial specifications and market requirements. Pesticides are used on lettuce to ensure high yielding, attractive products free from foreign bodies and damage. Pesticide reduction is a major challenge for lettuce growers in this context. The objective of this study was to assess the risk arising from a reduction in pesticide applications by using a combination of alternative techniques for the management of pests and diseases in winter lettuce crops. Two alternative crop protection strategies (called low-input and intermediate) were designed by prototyping and compared to conventional lettuce protection strategy in independent trials carried out in three locations during two years. The efficacy of each strategy for pest and pathogen control, as well as lettuce yield and quality, were assessed and compared. Pesticides were reduced by 32% in the intermediate crop protection strategy and by 48% in the low-input crop protection strategy. At least 15% of lettuces were affected by pest and pathogen damage, whatever the strategy. Aphids were the only pest or disease which differed significantly between strategies (9.25% of lettuce infested and 2.7% of commercial losses in the low-input strategy, compared to 0.83% of lettuce infested and 0% of commercial losses in the conventional strategy). Globally, biotic damage was less important than abiotic (frost and tip burn), and resulted in low commercial losses in every strategy. Similar yields and lettuce quality were observed in the three systems. Hence the performances of intermediate and low-input crop protection strategies were consistent with market expectations and their possible improvements are discussed.

Keywords

Lactuca sativa L., pesticides, prototyping, low-input, alternative techniques

1. Ce chapitre à été soumis à la revue *European Journal of Agronomy*

Abbreviations

CPS : Crop protection strategy, W : winter, L : location.

5.1 Introduction

Conventional farming systems use large amounts of pesticides to manage pest and pathogen populations. These are used to improve yield and visual quality of harvested products (Wilson and Tisdell 2001). However, due to the harmful effects of pesticides on the environment (Geiger et al. 2010 ; Goulson 2013) and possibly on consumers' and applicators' health (Mostafalou and Abdollahi 2013), the viability of conventional cropping systems is nowadays widely questioned. The European Union has recently established a directive to reduce pesticide consumption and promote the use, wherever possible, of non-chemical methods (European Union Parliament 2009).

Innovative cropping systems have been designed, mainly for arable crops, aimed at reducing pesticide use. During the last 20 years, a range of low-input cereal-based cropping systems have been designed and assessed (Loyce et al. 2012 ; Debaeke et al. 2009). These systems rely on the reduction of plant density, nitrogen inputs and sometimes tillage as well as the use of suitable, multi-resistant varieties (in mixtures or monocrop systems). Profit margins are maintained, since yield losses associated with reduced inputs are balanced by lower costs. In temperate and Mediterranean climates, winter lettuce is usually grown under shelter, typically in high tunnels, with two or three crops between September and April. In these systems, inputs are relatively marginal as compared to labor costs, and chemical protection represents only 3 to 6 % of the total production costs. The design of innovative lettuce based-cropping systems cannot therefore rely on the same methods as cereal-based systems, since yield losses cannot be offset by reduced input costs. Thus, any pesticide reduction in lettuce crops must be achieved without yield reductions. Furthermore, lettuce is usually eaten raw and the aerial parts of the plant are almost entirely consumed, so market specifications for visual quality and absence of foreign bodies are very high (Palumbo and Castle 2009). On average, the tolerance threshold of the industry for the presence of animal foreign bodies (including pests but also beneficial insects) is no more than 10% of products infested with no more than 5 individuals per item. Pesticides are therefore considered as an insurance to grow high-yielding and high-quality products. In organic agriculture, the possible yield reduction (de Ponti et al. 2012) can be balanced by a higher sale price of organic products, but many consumers are unwilling to pay these prices. Therefore, the question is how and how much is it possible to reduce the use of pesticides in conventional lettuce crops without affecting lettuce quality or yield.

Several pests and pathogens can threaten lettuce crops, such as biotrophic or necrotrophic, soil- or air- borne fungi, viruses, bacteria as well as aphids, moths, slugs, thrips, etc. (Blancard et al. 2006). Incidence and severity of each pest and pathogen depends on growing conditions (crop type, cultivation under shelter or in open fields, season) and change over the year. In winter lettuce crops under shelter, pathogens are predominant. The most important might be *Bremia lactucae* (Regel) that causes downy mildew, because of its rapid and devastating spread in the field. The pathogen may attack the plant throughout its life. The primary inoculum typically consists of airborne sporangia from diseased plants of the genus *Lactuca* located close to the crop or of mycelia present on plant debris in the soil (Crute 1992). *Sclerotinia sclerotiorum* (de Bary), *Sclerotinia minor* (Jagger), *Botrytis cinerea* (Pers.), *Rhizoctonia solani* (Kühn), and *Pythium tracheiphilum* (Matta) are other important fungal pathogens of winter lettuce crops. They are involved in the development of a shared symptom of basal rot (Van Beneden et al. 2009). *S. minor* and *S. sclerotiorum* are of major concern for the cultivation of lettuce because they may affect a wide range of plant species and their sclerotia may remain latent in the soil for more than 8 years (Melzer et al. 1997 ; Bolton et al. 2006). Moreover, sclerotia are often buried and dispersed due to tillage (Subbarao et al. 1996). Therefore, basal rot causes long-term problems in conventional lettuce crops since sclerotia are brought to the surface at each tillage. The fungus *Olpidium brassicae* (Woronin) is not a direct threat to lettuce, but a vector of two lettuce viruses that can cause significant damage, especially in winter : 'Mirafiori lettuce virus', responsible for

big vein disease ; and 'Lettuce big-vein associated virus', suspected to be the agent of ring necrosis (Lot et al. 2002 ; Verbeek et al. 2012). This fungus is able to remain dormant in the soil or on plant debris for several years in the form of chlamydospores (Blancard et al. 2006). Several aphid species can also occur in winter lettuce crops such as *Nasonovia ribisnigri* (Mosley), *Myzus persicae* (Sulzer), *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), and *Hyperomyzus lactucae* (L.). *N. ribisnigri* is the most damaging because it develops preferentially in the lettuce heart (Liu 2004). In addition to feeding damage and the loss of product quality due to their presence when the lettuce is marketed, aphids are also vectors of viruses, such as the lettuce mosaic virus. Finally, slugs (*Deroceras* sp. and *Arion* sp.) and snails can also cause feeding damage to lettuce during winter.

On average in conventional lettuce crops, eight pesticides are applied to manage pests and pathogens during the 60- to 90-day-long crop cycle. In winter, these are mainly fungicides. Due to the long time required before harvest for the elimination of active ingredients by lettuce and the lack of curative efficacy of pesticides for some pathogens such as *Bremia lactucae*, pesticides are almost exclusively applied preventively. Several alternative techniques with a partial effect on diseases and pests are currently available and can be combined to design innovative lettuce cropping systems less dependent on pesticides (Barrière et al. 2014). These alternative techniques are generally preventive and have only a partial effect on pests and diseases. Alternative techniques can act at different times in the pest and pathogen cycle : they i) can limit and reduce primary inoculum sources, ii) limit the development of pests and pathogens through the modification of the abiotic environment or iii) by increasing plant defenses and iv) have a curative action. Techniques that reduce primary inoculum in lettuce crops are sanitation methods such as the removal of infected plants, solarization or some biological control agents such as *Coniothyrium minitans* and *Trichoderma harzianum*. *C. minitans* can be used to reduce *Sclerotinia* spp., *B. cinerea*, and *R. solani* propagules (Chitrampalam et al. 2008). This fungus preferentially parasitizes overwintering structures by synthesizing chitinases, glucanases, and antifungal metabolites (Zeng et al. 2012). *T. harzianum* is also known to disturb populations of *B. cinerea*, *Pythium* spp., *R. solani*, and *Sclerotinia* spp. in soil, acting either as a competitor or as a parasite (Ozbay and Newman 2004).

Another way to protect lettuce crops is to limit the development of pests and pathogens through the modification of the abiotic environment. Indeed, the germination of infectious forms (spores or sclerotia) of numerous pathogenic fungi depends on climatic factors such as humidity and temperature. Drip irrigation, as opposed to sprinkler irrigation which is widespread in southern France, may reduce leaf wetness duration which is an important factor for the germination of *B. lactucae* sporangia (Scherm and Bruggen 1994) and *B. cinerea* spores (Elad and Shtienberg 1995). A reduction in planting density can also reduce foliage wetness due to better aeration, and limit soil-borne disease development by affecting the microclimate under lettuces.

Alternative techniques can also modify plant susceptibility to pests and pathogens. Genetic resistance, resistance inducers and nitrogen fertilization have shown a partial effect on lettuce diseases and pests. Lettuce varieties with major resistance genes against *B. lactucae* are available. However, resistance breakdown caused by new virulent strains of *B. lactucae* is common and leads to a rapid turnover of lettuce varieties (Michelmore and Wong 2008). Resistance to *N. ribisnigri*, due to the Nr gene, has also been introduced in many European varieties (Arend and Schijndel 1999), however this resistance has recently been circumvented by a new biotype named Nr :1. Also, different susceptibilities of lettuce accessions to *S. sclerotiorum* have been reported (Elia and Piglionica 1964 ; Grube and Ryder 2004 ; Hayes et al. 2010). In addition to genetic resistance, some techniques can strengthen plant defense. Several compounds, such as β-amino butyric acid or potassium phosphite (K_2HPO_3), have been identified as resistance inducers of lettuce against *B. lactucae* (Pajot et al. 2001). Some of them, such as potassium phosphite, also have a direct biocide effect on oomycetes (Massoud et al. 2012). Fertilization affects plant-pathogen and plant-pest interactions. Nitrogen fertilization has been shown to be positively correlated to *B. cinerea* or *S. sclerotiorum* damage on lettuce leaves (Lecompte et al. 2013). Leaf nitrogen content has also been positively correlated to lettuce palatability for slugs (Pakarinen et al. 1990). So, low nitrogen applications could help to manage those pathogens.

The efficacy of each alternative technique to pesticides is usually assessed separately, as a

stand-alone technique, and very few studies have investigated the effect of a coherent combination of alternative techniques on the control of the lettuce pest and pathogen complex (Collange et al. 2014). In this case, this is not a technique by itself, but the cropping system as a whole which is evaluated for its performance. Prototyping consists of the design, implementation and evaluation of innovative cropping systems, and allows theoretical constructs to be applied to production constraints (Vereijken 1997 ; Lancon et al. 2007). In order to adapt to local varying factors, techniques are described as a set of contingent decision rules that govern practices according to biotic and abiotic constraints (Debaeke et al. 2009 ; Papy 2001).

Field tests are necessary to assess the risks from reduced pesticide applications. In this study, combinations of alternative techniques and reduced applications of pesticides were designed to create alternative crop protection strategies (CPS) by prototyping, and compared to a reference strategy corresponding to current growers' practices. These CPS were implemented and their efficacy to manage pests and diseases, the yield and the quality of harvested products were evaluated in three locations during two seasons in order to cover the contextual variation of pest and pathogen pressure.

5.2 Material and Methods

5.2.1 Designing crop protection strategies

Fifteen persons including farmers, scientists, technical advisors, suppliers and end-product distributors participated in two expert meetings held in February and June 2011 in order to design CPS with reduced pesticide use. At the first meeting, the objectives and constraints of all the stakeholders were defined, and an inventory was made of possible alternative techniques to pesticides. Candidate CPS were then designed accordingly and refined during the second meeting, in which combinations of appropriate alternative techniques were validated and a target performance of each strategy identified. A first CPS, called low-input CPS, sharply reduced inputs and was intended to explore the technical boundaries of pesticide reduction. The second represented an intermediate level of pesticide reduction (intermediate CPS) between low-input CPS and current practices, and was intended to meet farmer's socio-economic and agronomic objectives. A conventional CPS, representative of current practices in protected winter lettuce crops, was also defined from the synthesis of 4 growers' practices.

5.2.2 Experimental design

The three lettuce CPS (conventional, intermediate and low-input) were used in Batavia lettuce production under high plastic tunnels in 3 locations in south-eastern France during winters 2012-2013 (W1) and 2013-2014 (W2). A total of 18 lettuce crops were observed (3 CPS x 3 locations x 2 years). Two locations were conventional production sites located at Candillargues (L1) (43 ° 62'N ; 4 ° 06'E ; 3 m elevation) and at Villelongue de la Salanque (L2) (42 ° 73'N ; 2 ° 98'E ; 6 m elevation) in the region Languedoc Roussillon. The third location (L3) was the experimental station of INRA located at Avignon (43 ° 91'N ; 4 ° 87'E ; 31 m elevation) in the region Provence Alpes Cote d'Azur (PACA). Crop rotation in each site, as well as planting and harvesting dates are given in Table 5.1. Crop lifespan was very similar for all experiments, between 70 and 84 days. The various combinations of location and years will be called L1-W1, L1-W2, L2-W1, L2-W2, L3-W1 and L3-W2.

5.2.3 Crop protection strategies

The innovative CPS relied on the joint implementation of alternative techniques to pesticides (Table 5.2). Pesticide applications and alternative techniques were managed by fixed or contingent decision rules to suit local constraints.

	L1	L2	L3
Place into rotation	S-L-L-M-S-L-L-M	S-L-L-L-S-L-L-bs	bs-L-L-S-bs-L-L
Planting date	12/10/2012 ; 12/05/2013	12/19/2012 ; 10/29/2013	01/07/2013 ; 01/15/2014
Harvesting date	02/26/2013 ; 02/24/2014	03/05/2013 ; 01/21/2014	03/18/2013 ; 03/28/2014

TABLE 5.1: Crop rotation and calendar of experimental plots. L : lettuce ; bs : bare soil ; S : solarization ; M : melon. Observed lettuce crops are underlined.

Technique	Conventional CPS	Intermediate CPS	Low-input CPS
Fungicide applications	Calendar-based	Calendar-based, including resistance inducer	Calendar-based, including resistance inducer
Insecticide applications	Regional alert	Presence in plots, with threshold	Presence in plots, with threshold
N fertilization	100 < soil N content < 120 kg.ha^{-1}	100 < soil N content < 120 kg.ha^{-1}	Reduced and split applications
Irrigation	Sprinkler	Sprinkler	Sprinkler or drip
Planting density.m^2	14.25	14.25	12.75
Genotype	Notilia	Ostralie	Lasydo
Biological control of pathogens	*C. minitans*	*C. minitans*	*C. minitans* and *T. harzianum*
Biological control of aphids	No	Parasitoïds mix	Parasitoïds mix
Infected plant management	No	No	Removal

TABLE 5.2: Technical operations for each CPS.

Techniques that affect several pests and pathogens

Irrigation Except for the plot cultivated under low-input CPS in L3, all the plots were sprinkler-irrigated throughout crop growth. Just after planting, water was applied to field capacity. The moisture content was maintained in the balls containing the plantlets by short daily irrigations until the roots started to grow into the soil. After this early growth stage, drip irrigation was used under low-input CPS in L3 only. Irrigation was triggered according to the soil water potentials measured by 6 Watermark®sensors placed at 3 positions and 2 depths (15 cm and 35 cm), following the current guidelines in SE France (Bressoud 1998). At the other sites and CPS, sprinkler irrigation was triggered once every two or three weeks, according to ETP. Towards the end of crop growth, short sprinkler irrigations were triggered when the temperature exceeded 30 ° C, to allow rapid cooling of the lettuces.

Fertilization A few days before planting, about fifteen 30 cm deep soil samples were randomly collected in each plot. Soil nitrate content was measured for each plot with a Nitrachek reflectometer. Nitrogen fertilization was calculated from the results of these analyses. With conventional CPS and intermediate CPS, inputs of mineral nitrogen were applied to adjust the nitrate content between 100 and 120 kg NO3 -N.ha^{-1}. If nitrate content exceeded 120 kg.ha^{-1} prior to planting, no fertilizer was applied. For plots cultivated under low-input CPS, nitrate fertilization was reduced and split. Before planting, soil nitrate-N content was adjusted to 40 kg.ha^{-1}. At the 16^{th} leaf stage, a new soil sample was collected and analyzed following the same procedure, and, using this updated measurement, the soil nitrate-N content was adjusted to 60 kg.ha^{-1}. Due to significant N mineralization during early growth, the soil nitrate stock at this stage was usually not exhausted, resulting in low fertilizer applications. As a result, the total application of N fertilizers in the plots cultivated under low-input CPS was much lower than in the two other CPS.

Genotype A different cultivar was used in each CPS. Cv Notilia (Clause), used in conventional CPS, is a fast-growing variety and has almost a complete range of resistance to *B. lactucae* (Bl 1-28, 30, 31). Cv Ostralie (Rijk Zwaan), was used in intermediate CPS. This cultivar grows slightly more slowly, has a complete range of resistance to *B. lactucae* (Bl : 1-31) and has a semi-upright habit. In the low-input CPS, Lasydo (Syngenta seed), is a fast-growing variety with an almost complete range of resistance to *B. lactucae* (Bl 1-28, 30, 31) and a low susceptibility to *S. sclerotiorum* (cf. Chapitre 3).

Planting density Lettuces were planted in parallel rows, on micro- and macro- perforated black plastic mulch (Optimac®). The planting density in low-input CPS was reduced from 14.25 to 12.75 plants per square meter.

Infected plant management In low-input CPS, any lettuce showing pathogen damage rendering the plant unmarketable was carefully removed from the plot in order to prevent inoculum dissemination.

***B. lactucae* management**

Fungicide applications All fungicides were applied with a spray boom, following a calendar-based program. In plots cultivated under conventional CPS, two applications of Infinito®(Bayer) (at 1.6 $l.ha^{-1}$) and two applications of Sygan®(Dupont) (at 2.5 $kg.ha^{-1}$) were applied against *B. lactucae* 8, 30 and 20, 40 days (± 2 days) after planting, respectively. In plots cultivated under intermediate and low-input CPS, the number of fungicide applications was reduced : one application of Infinito®(Bayer) (dose :1.6 $l.ha^{-1}$) and one application of Sygan®(Dupont) (at 2.5 $kg.ha^{-1}$) against were made 10 and 32 days (± 2 days) after planting, respectively.

Resistance inducer For the intermediate and low-input CPS, the treatments with fungicides were supplemented by applications of a plant resistance inducer (Potassium phosphite – LBG 01F34®) (rate : 2 $l.ha^{-1}$) applied by spraying 22 and 44 days (± 2 days) after planting.

Basal rot management

Biological control In all the CPS, one application on the soil surface of the fungus *Coniothyrium minitans* (Contans®) at a rate of 2 $kg.ha^{-1}$ was made before the first lettuce crop (in October). A second application was carried out before planting in intermediate- and low-input CPS. Additionally, for low-input CPS, the biocontrol fungus *Trichoderma harzianum* (strain T22 – Trianum P®) was applied twice on the balls, at the cotyledon stage (at 1.5 $g.m^2$) and just prior to planting (at 1 kg per 8500 plants). The presence of *Trichoderma* spp. on lettuce roots was assessed 30 days after planting.

Fungicide applications On plots cultivated under conventional CPS, one application of Signum®(BASF Agro) (at 1.5 $kg.ha^{-1}$), one application of Switch®(Syngenta) (at 0.6 $kg.ha^{-1}$) and one application of Rovral®(BASF Agro) (at 1 $kg.ha^{-1}$) were made at 8, 20 and 30 days (± 2 days) after planting, respectively. In plots cultivated under intermediate- and low-input CPS, the number of fungicide applications was reduced. One application of Signum®(BASF Agro) (at 1.5 $kg.ha^{-1}$) and one application of Switch®(Syngenta) (at 0.6 $kg.ha^{-1}$) were made on plots cultivated under intermediate CPS at 10 and 32 days (± 2 days) after planting respectively while, for the low-input CPS, only one application of Signum®(BASF Agro) (at 1.5 $kg.ha^{-1}$) was made 20 days (± 2 days) after planting.

Aphid management

Biological control of aphids Mixtures of parasitoid species (Basilprotect®) including *Aphidius colemani*, *Aphidius ervi*, *Aphidius matricariae*, *Aphelinus abdominalis*, *Aphedrus cerasicola* and *Praon volucre* were preventively introduced at the center of each plot cultivated under intermediate CPS and low-input CPS at a rate of 1.2 individuals per m^2 every two weeks.

Insecticide application For the conventional CPS, one insecticide was applied every seven days as soon as aphids were observed in the area of production. For intermediate and low-input CPS, insecticide treatments were triggered when the number of aphids had reached species-dependent thresholds. The count was made on 40 lettuces per plot, randomly selected every week. The threshold for *N. ribisnigri* was 2% and 10% of lettuce plants infested in plots under intermediate CPS and low-input CPS respectively. For other aphid species, it was 5% and 15% respectively.

5.2.4 Performance assessment

Pesticide use

The Treatment Frequency Index (TFI) (Gravesen 2003 ; Champeaux 2006) was used to assess the amount of pesticide applied in each CPS. TFI accounts for the number of compounds, the number of treatments and the rate applied per unit area, and is calculated as follows :

$$TFI = \sum \frac{\text{Applied rate} \times \text{Area treated}}{\text{Authorized minimal dose} \times \text{Plot area}}$$

Monitoring of pests and pathogens

In France, since the implementation of the Ecophyto program (Ecophyto 2008), *i.e.* the government plan to reduce pesticide use, pest and pathogen monitoring networks have been created. They aim to assess the epidemiological risks and to broadcast agricultural warning reports for each crop in each region. Regional agricultural warning reports for lettuce, broadcast in the PACA and Languedoc Roussillon regions about every 15 days, were used in this study to determine timing of the preventive insecticide applications in the plots cultivated under conventional CPS and to appraise pest and pathogen pressure during the experiments. The presence of pests and pathogens in the experimental plots was assessed weekly on 40 plants selected at random at each date. 60 plants per plot were collected at harvest. The percentage of lettuces showing damage was recorded. The damage caused by each pest or pathogen or by an abiotic cause was scored from 1 to 3 (Table 5.3). A score of 1 at the time of harvest indicates no effect on sale ; a score of 2 affects the lettuce quality without preventing sale, and with a score of 3, a lettuce is unsaleable.

Lettuce yield and quality

At harvest, 60 lettuces for each CPS were randomly sampled and used to assess production performance of each CPS. The percentage of marketable lettuces (%ML), the average lettuce fresh weight (LW, g), the marketable weight (after the removal of unmarketable basal leaves,

Pest or pathogen	1: no effect	2: effect on quality	3 : unmarketable lettuce
Aphids	< 5	5 < aphids < 9	> 10
Basal rot agents	Collar mark	Symptoms on 1 or 2 basal leaves	More symptoms
B. lactucae	/	1 to 2 lesions	> 2 lesions
Slugs	Slight damage on basal leaves	Severe damage on basal leaves	More damage
Damage with abiotic cause (Tip burn, frost, etc.)	Bursting of central ribs of 1 or 2 basal leaves	Symptoms on basal leaves	Symptoms on other leaves

TABLE 5.3: Scoring for damage caused by lettuce pathogens and pests and growing conditions.

MLW, g), and the percentage of unmarketable basal leaves were measured. The gross yield (t fw.ha^{-1}) was calculated as :

$$\text{Gross yield} = \frac{LW \times \text{planting density}}{100}$$

The marketable yield (t fw.ha^{-1}) was calculated as :

$$\text{Marketable yield} = \frac{MLW \times (\%ML) \times \text{planting density}}{100}$$

Ten marketable lettuces were randomly harvested in each plot for a visual quality assessment based on plant appearance and absence of foreign bodies. The ratings were 0 (very poor quality), 1 (poor quality), 2 (middling quality), 3 (good quality), 4 (very good quality), 5 (excellent quality).

5.2.5 Data analysis

How to analyze the efficacy of CPS ?

The incidence of damage in a cultivated plot reflects both the efficacy of CPS, the presence of pests and pathogens and of favorable conditions for their development. To analyze the efficacy of CPS, damage structure caused by each pest or pathogen was observed. Six types of structures could be observed (Table 5.4). Those structures will be used to organize the 'Results' section and guide the discussion. The damage structure in all the experimental plots, together with several hypotheses about pest or pathogen pressure, enabled us to draw conclusions about the efficacy of each CPS. CPS could be efficient under all experimental conditions, only for some conditions, or simply inefficient. In the absence of pest pressure, no conclusions could be drawn about the efficacy of CPS.

Statistical analyses

Considering the small size of the sample (18 values per variable), non-parametric Kruskal-Wallis rank sum tests (with a significance threshold of 0.1) were used to analyze the effect of CPS on each performance indicator. To test for the effect of CPS on a particular pest or pathogen incidence, only the locations where the pest/pathogen was observed were kept for the Kruskal-Wallis rank sum test. Rank-based multiple comparison test (De Mendiburu 2009) were used when significant differences among CPS, locations or winters were found. All statistical analyses were carried out with R (R-Core-Team 2013).

5.3 Results

5.3.1 Pesticide reduction

The TFI differed significantly between CPS (P= 0.002794) (Figure 5.1). On average, pesticides were reduced by 31.8% in intermediate CPS and by 47.7% in low-input CPS. Fungicides were reduced by 43.1% and 56.7% in intermediate and low-input CPS respectively while insecticides were reduced by 55.3% and 78.0%. The lower pesticide use was partly balanced by resistance inducers which are accounted for in TFI, and resulted in a mean increase of 1.14. No significant effect of either location or year was found.

5.3.2 Efficacy of pest and pathogen management

Unobserved damage

Several pests and pathogens with frequent occurrence in winter lettuce crops were not observed in this study. This is the case of *B. lactucae* and viruses transmitted by *O. brassicae*. However, *B. lactucae* was mentioned in 2/5 and 3/4 of the agricultural warning reports during

DAMAGE STRUCTURE		EXAMPLE	
All CPS without damage		W1	W2
		L1 C I L	C I L
		L2 C I L	C I L
		L3 C I L	C I L
All CPS with damage at all locations		W1	W2
		L1 C I L	C I L
		L2 C I L	C I L
		L3 C I L	C I L
Some CPS with damage at some locations	Some CPS without damage	W1	W2
		L1 C I L	C I L
		L2 C I L	C I L
		L3 C I L	C I L
	All CPS with damage at least at one location	W1	W2
		L1 C I L	C I L
		L2 C I L	C I L
		L3 C I L	C I L
All CPS with damage at some locations		W1	W2
		L1 C I L	C I L
		L2 C I L	C I L
		L3 C I L	C I L
Some CPS with damage at all locations		W1	W2
		L1 C I L	C I L
		L2 C I L	C I L
		L3 C I L	C I L

TABLE 5.4: Six possible types of damage structure in the experimental plots implemented in three locations (L1, L2 and L3) during two winters (W1 and W2). CIL refers to conventional, intermediate and low-input CPS. In the examples, the underlined letters indicate plots with damage.

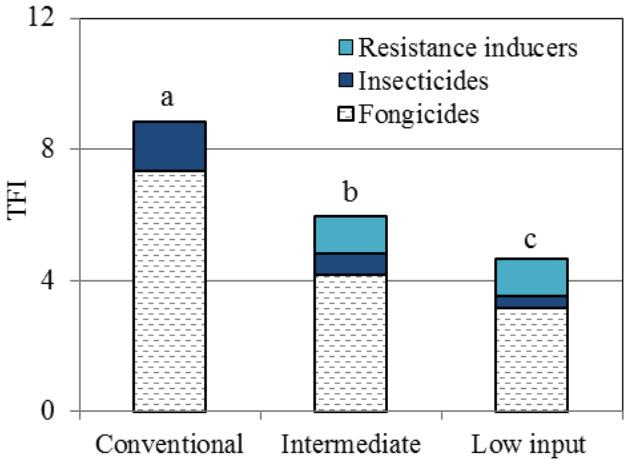

FIGURE 5.1: Plant resistance inducer, insecticide and fungicide contributions to Treatment Frequency Index (TFI) in the different crop protection strategies. Results are given as the mean of 3 locations and 2 years. Different letters indicate significantly different values in the multiple comparison tests.

CPS	L1-W1	L1-W2	L2-W1	L2-W2	L3-W1	L3-W2	Average
Conventional	5.0^{Mp}	0	0	0	0	0	0.83 a
Intermediate	0	0	1.7^{Mp}	0	0	6.3^{Nr}	1.33 a
Low-input	26.6^{Mp}	1.7^{Mp}	8.3^{Mp}	0	0	15.0^{Nr}	9.25 b

TABLE 5.5: Percentage of lettuces infested by aphids at harvest in the three CPS and three locations (L) during both winters (W). Superscripts represent the aphid species observed, Mp : *Myzus percicae*, Nr : *Nasonovia ribisnigri*. In the « average » column the letters 'a' and 'b' indicate the result of Kruskal–Wallis tests.

W1 in the PACA and Languedoc Roussillon regions respectively and in half of the warning reports during W2 in both regions. Viruses transmitted by *O. brassicae* were reported only in the PACA region and in 1/5 of the reports during winters 1 and 2.

Presence of damage in some locations, in some CPS : Aphids

Aphids were found in 8 of the 18 experimental plots. Two species were observed : *N. ribisnigri* and *M. persicae*. The lettuces of the low-input CPS were significantly more infested than those of intermediate and conventional CPS (P= 0.053) (Table 5.5). No significant difference was found between winters or locations. Aphids caused 0, 0.9% and 2.7% of unmarketable lettuces at harvest in conventional, intermediate and low-input CPS plots respectively.

Population dynamics in the most infested plots L1-W1 and L3-W2 are reported in Figure 5.2. Some of the variability observed from one sampling date to another might be explained by an uneven dispersal of aphids in the fields. Nevertheless, some patterns can be distinguished. In L1-W1, the field interventions were identical in the plot cultivated under low-input and intermediate CPS, consisting of preventive introductions of parasitoids without insecticide application. In the conventional CPS, one insecticide (Movento®- Bayer) (at 0.75 $l.ha^{-1}$) was applied preventively. This single application failed to provide complete control, since aphids were observed on 5% of the plants at harvest. Despite the identical management in low-input and intermediate CPS, the percentage of lettuces infested by *M. percisae* was greater in the low-input CPS, especially at harvest (Figure 5.2(a)). However, the severity was generally higher in the intermediate CPS, which might indicate that aphids were less mobile in this treatment. Although the density was lower under low-input CPS, the cultivar had higher growth rates, and there was more contact between leaves of adjacent plants at the end of growth, which might have favored dispersal. In the low-input CPS, 7.8% of aphids were parasitized at harvest while no mummified aphids were observed in the plot cultivated under intermediate CPS.

N. ribisnigri was observed only in L3 during W2 and only in intermediate and low-input CPS (Figure 5.2(b)). In the conventional CPS, three insecticides were applied. In alternative CPS, parasitoids were introduced preventively, but the threshold for *N. ribisnigri* infestation was reached on three sampling dates in the intermediate CPS plots, and on one sampling date in the low-input CPS plots, leading each time to curative applications of pesticides. These treatments failed to control the aphids. As in L1-W1, the severity was higher in the intermediate CPS plots, while the percentage of infested lettuces increased faster in the low-input CPS plots. But in this case, the different number of insecticide treatments probably explained the different aphid dynamics. 3.6% and 15.4% of aphids were parasitized at harvest in intermediate and low-input CPS respectively.

Presence of damage in all CPS in some locations

Basal rot Basal rot symptoms were observed in all CPS at some locations in at least one of the two winters (Table 5.6). However, damage was limited, since fungal rots were usually observed on the lettuce collar and/or the oldest leaves, and removed at harvest. On average, 92% of lettuces with basal rot symptoms were marketable. Significant differences in basal rot incidence were

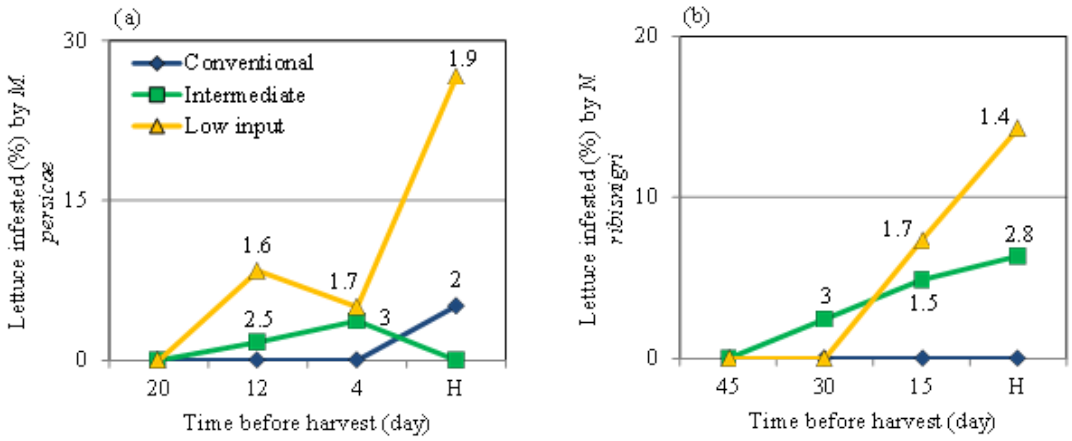

FIGURE 5.2: Dynamic of the number of lettuces infested by *M. persicae* in L1-W1 (**a**) and *N. ribisnigri* in L3-W2 (**b**) in plots cultivated under conventional, intermediate and low-input CPS. The numbers are the severity scores.

CPS	L1-W1	L1-W2	L2-W1	L2-W2	L3-W1	L3-W2
Conventional	1.7	21.7	3.3	45.0	0	18.3
Intermediate	0	3.3	1.7	26.7	0	0
Low-input	0	3.3	6.7	73.3	0	11.7

TABLE 5.6: Percentage of lettuces showing basal rot symptoms at harvest in the three CPS and three locations (L) during two winters (W).

observed between locations (P= 0.070) and between winters (P= 0.005) but not between CPS (P= 0.3379). No CPS, location or winter had a significant effect on basal rot severity.

Infected and healthy lettuce weights were compared at harvest in plots where basal rot incidence was over 15% (Figure 5.3). The weight of infected lettuces was higher than the weight of healthy lettuces in three of them, while no significant difference was observed in the other plots. Some factors may explain the absence of significant differences between healthy and infected lettuces grown under conventional CPS in L1-W2 and under intermediate CPS in L2-W2. Lettuces grown under conventional CPS in L1-W2 had similar weights (standard deviation ± 72) as those in the other plots (± 149). In L2-W2, weights of both infected and healthy lettuces might be too low to induce differences in disease incidence.

Slugs and snails The CPS did not have a significant impact on the incidence of slugs and snails, nor on the severity of the damage (P= 0.7605). However, a significant location effect was found (P= 0.055). Slugs and snails were particularly numerous in L2 during W1 and in L3 (Table 5.7). They caused major losses, since on average 19.9% of lettuces attacked were unmarketable.

CPS	L1-W1	L1-W2	L2-W1	L2-W2	L3-W1	L3-W2
Conventional	0	6.7	30.0	0	11.7	21.7
Intermediate	0	1.7	28.3	0	10.0	17.5
Low-input	0	6.7	65.0	1.7	23.3	20.0

TABLE 5.7: Percentage of lettuces showing slug or snail damage at harvest in plots cultivated under the three different CPS and three locations (L) during both winters (W).

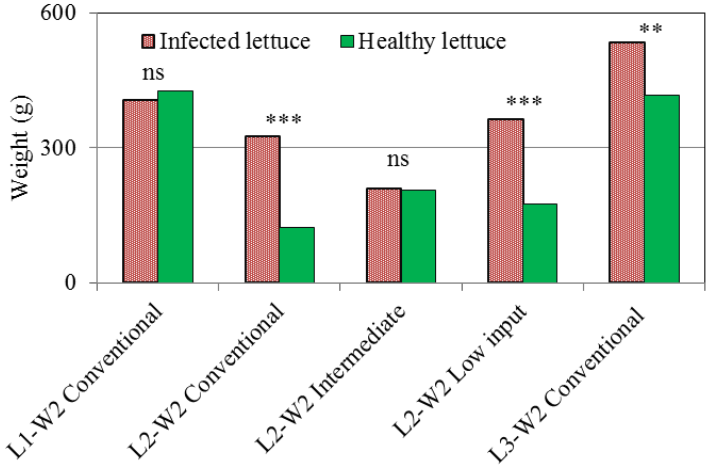

FIGURE 5.3: Weight of infected and healthy lettuces in plots that had more than 15% of plants attacked by basal rot causal agents. Results of the Kruskal-Wallis rank sum test are given as 'ns' : not significant, '$**$' : P <0.01, '$***$' :P <0.001.

Overall pest and pathogen damage

In summary, pest or disease damage was observed in all CPS (Figure 5.4(a)). The overall pest and pathogen incidence differed among the CPS ; however, these differences were only significant between low-input and intermediate CPS (P= 0.07783). The conventional CPS showed intermediate pressure, and did provide more effective protection than the treatments with less pesticide. The overall pest and disease incidence also varied sharply between locations (P= 0.02606) (Figure 5.4(b)).

Other damage

Frost and tip burn (marginal necrosis due to calcium deficiency favored by incorrect water supply) damage were also repeatedly observed. Abiotic damage accounted for 64%, 41% and 26% of total damage in L1, L2 and L3 respectively. Tip burn incidence was influenced by CPS (P= 0.03177) while frost damage depended more on location (P= 0.0345). As a whole, these abiotic forms of damage caused 9% of commercial loss, *i.e.* more than the 5% of loss due to pest and pathogens.

Yield and lettuce quality

The percentage of marketable lettuces, the marketable lettuce weight, the gross yield and the marketable yield were not significantly influenced by CPS or by location. The average marketable yield was 37.40 t.ha^{-1} ($\pm$ 12.68). The percentage of unmarketable basal leaves at harvest differed between locations (P= 0.06477), but not between CPS. However, the CPS affected the quality rating (P= 0.01131) (Table 5.8). The lettuces with the best appearance were harvested from plots cultivated under intermediate CPS while the lowest visual quality was observed in the conventional CPS plots.

5.4 Discussion

Some of the main lettuce winter pathogens were not observed in this study. The low pressure exerted by viruses transmitted by *O. brassicae* meant that we could not draw conclusions about the efficacy of alternative techniques. However, the high pressure of *B. lactucae* reported in the production area during winters 2012-2013 and 2013-2014 and the extensive distribution of this

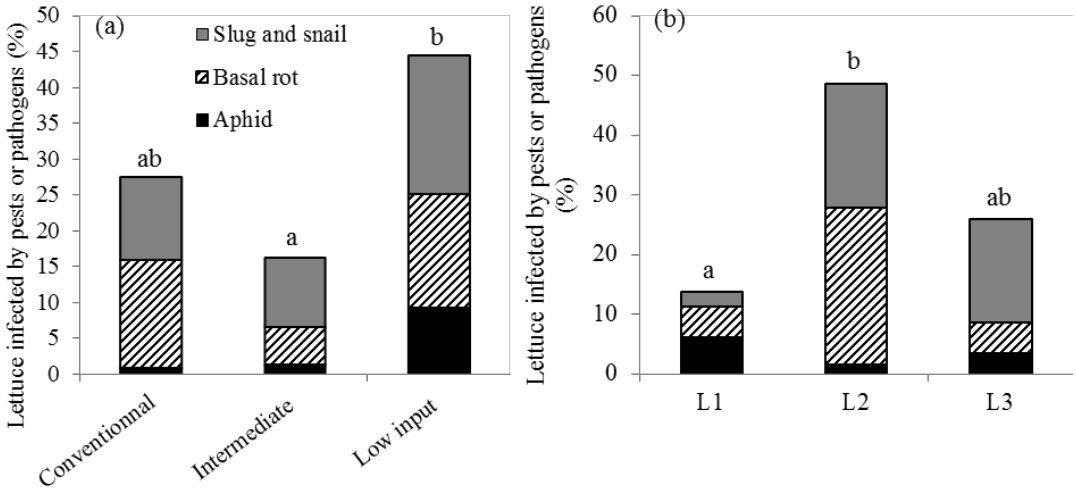

FIGURE 5.4: Numbers of lettuces (%) attacked by aphids, basal rot causal agents or slugs and snails in conventional, intermediate or low-input CPS (**a**) and in locations L1, L2 and L3 (**b**). Letters indicate the result of Kruskal–Wallis tests.

	MLW (g)	ML (%)	Marketable yield (t.ha^{-1})	Quality rating
Conventional	362	71.4	36.34	1.65 a
Intermediate	336	82.9	39.88	2.80 b
Low-input	359	78.3	35.99	2.00 a
P- value	ns	ns	ns	< 0.05

TABLE 5.8: MLW, ML, marketable yield and quality rating of lettuces cultivated under conventional, intermediate and low-input CPS. P-values are the result of Kruskal-Wallis rank sum test.

pathogen in France (Valade et al. 2010) suggest that all three CPS may have provided efficient protection against this pathogen. Genetic resistances to *B. lactucae* rely on specific genes for genes interactions. Additional techniques, such as fungicide applications, are usually used to ensure the sustainability of genetic resistance and the efficacy of *B. lactucae* control (Crute 1984). In the intermediate and low-input protection strategies against *B. lactucae*, preventive fungicide applications have been partly replaced by plant resistance inducers. Consequently, the combination of genetic resistance, reduced fungicide applications and resistance inducers might be an effective strategy to manage *B. lactucae*. Additional experiments should be carried out to reinforce these conclusions over a broader range of cropping situations.

Some lettuce pests were observed only in some locations and some CPS. For instance, aphids were observed in 44% of the plots. Aphid damage was the only biotic pressure significantly affected by CPS. However, aphids were observed in every CPS, so none of the strategies was completely effective. In the conventional CPS, the preventive insecticide applications made when aphids were observed in the production area resulted in no or few aphids at harvest both for *N. ribisnigri* and *M. persicae*, so chemical control was moderately efficient. In L1-W1, the preventive introductions of parasitoids in plots cultivated under low-input and intermediate CPS helped to maintain *M. persicae* populations below the treatment thresholds. In low-input CPS, a higher proportion of lettuces contained aphids, while the number of aphids per plant was higher in intermediate CPS. This difference might have been mediated by the plants. Indeed, several studies have shown different susceptibilities to aphids among lettuce cultivars (Dunn and Kempton 1980 ; Lu et al. 2011). Furthermore, studies on *Brassica* sp. showed that aphid populations increase with narrower plant spacing (Sarwar 2008 ; Yamamura 1999). In our work, the planting density was greater but the lettuce spacing wider in the intermediate CPS, since the cultivar showed a moderate growth rate, when compared to that of low-input CPS. Wider spacing among lettuces might have impeded the spread of aphids.

Due to the industrial requirements for visual quality and absence of foreign bodies, Palumbo and Castle (2009) have argued that the introduction of biological control agents is not feasible in lettuce crops. Here we have shown that preventive introductions of parasitoids could, in certain situations, help to maintain the *M. persicae* population below the threshold for chemical intervention (5% and 15% of lettuces infested respectively for intermediate and low-input CPS) and provide high quality products. However, in the case of invasions of *N. ribisnigri* (as in L3-W2), the preventive introduction of parasitoids failed to maintain populations below the treatment thresholds of 2% and 10% of lettuces infested for intermediate and low-input CPS respectively for either of these CPS. This aphid develops preferentially in the lettuce heart (Liu 2004), which may hinder its detection by parasitoids. It is known to be not susceptible to contact insecticides (Liu 2004 ; Mackenzie and Vernon 1988), so systemic insecticides were used in this study. Their preventive applications in the conventional CPS ensured good protection. However, the curative efficacy of the same insecticides was not as good, since three insecticide applications did not suffice to suppress aphids totally. These results suggest that lower treatment thresholds might be required for *N. ribisnigri*. A more stringent threshold was already defined by Morales et al. (2013) for *N. ribisnigri* as 0.06 and 0.07 aphids per lettuce respectively for field-grown lettuce during spring and autumn in central Spain. Those thresholds could be used for winter lettuce grown under shelter.

Basal rot was observed in all CPS in some locations in this study. The three strategies were equivalent in terms of damage, although they relied on different control techniques. Only a few alternative techniques were added in the intermediate CPS (biological control agent and a semi-upright lettuce habit) to reduce fungicide applications by one third. The fact that lettuce weight had influenced basal rot incidence could partly reflect the effect of microclimate under lettuces on basal rot incidence. The wide plant spacing and upright lettuce habit in the intermediate CPS might have reduced the humidity below the plants and limited basal rot development. In the low-input CPS, several techniques known to partially limit the damage caused by basal rot (biological control agents, sanitation method, reduced N fertilization, cultivars with reduced susceptibility to *S. sclerotiorum*, reduced planting density and, in one case, drip irrigation) were used to reduce the number of chemical treatments against basal rot by two-thirds. All of them had shown a partial effect on at least one of the causal agents of basal rot in factorial experiments

(Chitrampalam et al. 2008 ; Lecompte et al. 2013 ; Dow et al. 1988). However, the combination of some alternative techniques may have induced negative synergy since little information was available on the interaction between techniques. Field experiments enabled us to assess CPS efficacy but did not permit us to fully understand synergy and antagonism among techniques. The techniques that were most likely to interact with each other were those acting at the same point of the pathogen's life cycle. For example, two alternative techniques were used in the low-input CPS to increase lettuce defense : the use of a lettuce variety with low susceptibility to *S. sclerotiorum* and the reduction in nitrogen input. The combination of these two techniques may have reduced their individual effects on basal rot agents. Therefore, specific experiments combining lettuce varieties with different levels of susceptibility to *S. sclerotiorum* and different levels of N fertilization should be performed to understand the interaction between these factors.

Some of the alternative techniques used in the low-input CPS might have long-term effects or improved efficacy after several seasons of application. Here we assessed the incidence and severity of basal rot during two winters, which is not long enough to evaluate such effects. Among these alternative techniques, the introduction of *C. minitans*, which parasites *Sclerotinia* spp. sclerotes could have an impact on soil inoculum (Chitrampalam et al. 2008).

In these six implementations, we demonstrated that, except for aphids, the pest and pathogen complex might be managed with less pesticide. According to these first results, some improvements in the CPS can be suggested, such as using a lower treatment threshold for *N. ribisnigri*. Also, since close plant spacing appeared to favor both the development of basal rot and aphids, we suggest planting a compact lettuce variety, i.e. small but heavy to maintain yield. Moreover, in this study, much of the damage was due to abiotic stresses such as frost and tip burn. The design of alternative CPS was centered on potential biotic damage. One way to improve the CPS would be to take into account the potential abiotic damage. For example, varietal choice should include tip burn resistance, especially when drip irrigation is used. In general, interactions between variety and cultural practices may influence lettuce quality, number of pesticide residues, lettuce weight, etc. Perhaps because of the rapid turnover of lettuce varieties, this information is seldom available, and therefore it is critical to pay particular attention to variety/practice interactions during the design process.

Despite the stringent market requirements in terms of visual quality and the need to maintain yield, pesticides were reduced by half in the low-input CPS. This reduction is consistent with those obtained on other low-input cropping systems designed by prototyping (Clark et al. 1998 ; Simon et al. 2011). The marketable yield was not affected by CPS. The higher proportion of marketable lettuces with the low-input CPS has partially offset the lower planting density, explaining its similar yields to the other CPS. The prototyping method has enabled crop protection strategies to be designed which are compatible with market expectations and production constraints. The possible environmental gains of intermediate and low-input CPS could be significant and should be assessed together with socio-economic performance in order to encourage the adoption of alternative strategies by lettuce growers.

5.5 Acknowledgments

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Chapitre 6

Environmental and economic performances of lettuce crops designed to reduce pesticide use [1]

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Abstract

Facing environmental and public health issues, pesticide reduction is a short term objective shared by European governments and fruit and vegetable purchasers. In lettuce crops, several alternative techniques, with partial effect on pests and pathogens might be integrated in innovative crop protection strategies (CPS) to reach this objective. More than their efficacies, the economic and environmental performances of alternative strategies need to be determined to enhance their adoption by farmers. In this study, two alternative lettuce protection strategies (called low-input and intermediate) were designed by prototyping and compared to conventional lettuce protection strategy in independent trials carried out in three locations during two years. The economic performance was evaluated through the production cost and profitability limit calculation while indicators of environmental performance were the Environmental Impact Quotient (EIQ) and the abundance and diversity of epigeic macro-arthropods. Total production costs were 30 984 €. ha^{-1}, 35 105 €.ha^{-1} and 33 986 €.ha^{-1} in the conventional, intermediate and low-input CPS, respectively. Additional costs in the alternative strategies most exclusively relied on biological control methods. For similar yields, the increase of lettuce sale price by 13% in the intermediate CPS and by 10% in the low-input CPS would maintain profitability as in the conventional CPS. The average total EIQ were 207, 159 and 95 for the conventional, intermediate and low-input CPS, respectively and the total abundance of arthropods was significantly higher in the alternative strategies, showing the potential environmental gains from pesticide reduction. The adoption of alternative CPS now relies on a reduction of biological control cost or on the creation of additional market segmentation with a slightly increase of selling prices.

Keywords

Crop protection, yield, pesticide residues, production costs, arthropods abundance

1. Ce chapitre sera prochainement soumis à la revue *Agronomy for Sustainable Development*

6.1 Introduction

Lettuce (*Lactuca sativa* L.) is a leafy vegetable produced worldwide in temperate and subtropical areas (Mou 2008), grown in open field or under shelters. The pool of pests and pathogens that threaten lettuce crops depends on location and season. Under cool and moist conditions, major threats are fungal pathogens such as *Bremia lactucae* (Regel), *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* Jagger ; while several pests (e.g., aphids, moths, thrips, slugs, etc.) can cause damage in warmer and drier conditions. In conventional lettuce production, pests and pathogens are mainly managed by preventive applications of pesticides which generally represent no more than 6 % of the total production costs. Alike other fruits and vegetables, lettuce has to meet cosmetic standards (Pimentel et al. 1993), but this is exacerbated in the case of lettuce which is a leafy vegetable consumed almost entirely. To ensure high yields and high visual quality of products, pesticides are currently applied preventatively. Nevertheless, due to their potential harmful impacts on the environment and on human health, reducing the use of pesticides has become a major issue of agriculture. In the European Union, the directive 2009/128/CE (2009), encouraging pesticide reduction, was established in 2009. As a result, several actions have been implemented in EU countries such as the Ecophyto plan in France (2008) which aims to reduce pesticide use by 50% by 2018. Moreover, maximal limit of pesticide residues (MLR) have been implemented to mitigate the impact of pesticide on consumer health. The requirements of lettuce purchasers (supermarket and industry of ready-to-eat salads) in terms of MLR are often more restrictive than legal ones. Hence, the most demanding markets can also be major drivers of pesticide reductions (Cameron 2007).

In order to reduce pesticide applications, agricultural practices should be reconsidered. Several alternative techniques, with partial effect on pests and pathogens, might be integrated in alternative crop protection strategies (CPS) (Debaeke et al. 2008 ; Barrière et al. 2014). Although, numerous factors govern the adoption of alternative techniques by farmers, a prominent factor is the economic return. In organic agriculture, the eventual yield reduction due to the absence of chemical control, (de Ponti et al. 2012) can be balanced by higher sale price of organic products, which may be twice the price of conventional products (Boccaletti and Nardella 2000). However, this economic model may not be suitable for all consumers. Thus, in the absence of market segmentation for lettuces cultivated with less pesticides, alternative strategies to manage lettuce pests and pathogens should show efficacy and production costs as close as possible of those of conventionnal strategies.

Moral and social concerns can also influence the adoption of alternative practices by farmers (Mzoughi 2011). In particular, an evaluation of the environmental impact of pesticide reduction could help to promote the adoption of alternative techniques to pesticides. The environmental impact is one of the three factors (with the economic and social impacts) characterizing the sustainability of cropping systems (Lichtfouse et al. 2009). The environmental performance of a crop protection strategy could be defined as the ability to maintain agroecosystem functions, such as nutrient recycling, pollination, nitrogen fixation, population regulation, etc. Arthropods are among the organisms that are able to ensure these functions, especially pollination and natural population regulation (Olfert et al. 2002 ; Losey and Vaughan 2006). Agricultural practices may affect the abundance and the diversity of beneficial organisms and, thus, can affect the environmental performances. For example, pesticide applications, as well as other practices in intensive cropping, can adversely affect the biodiversity of natural enemies of pests and induce an overall increase of pests abundance (Altieri 1999). Several indicators have been developed to assess environmental performances of agroecosystems at the landscape, farm or plot scales (Bockstaller et al. 1997 ; Van der Werf and Petit 2002). They can be based on the means used by farmers or on the effect of these means on environment. Some of the means-based indicators focus on pesticide use such as the Treatment Frequency Index (Gravesen 2003 ; Champeaux 2006) which calculates the amount of pesticide applied, or the Environmental Impact Quotient (EIQ) (Kovach et al. 1992) which estimates the toxicity of the set of pesticide applied on human health and fauna.

Currently, without information describing the efficacy, the cost and the profitability of alternative protection strategies, producers who want to change their practices are moving more towards organic farming perceived as easier to implement (Lechenet et al. 2014). This fact emphasizes

the need to investigate and describe the agronomic, economic and environmental consequences of crop protection strategies that partially substitute pesticides by other techniques (Clark et al. 1998).

This work provides a case-study on winter lettuce grown under shelter in the Mediterranean area for the industrial market Two CPS were designed and tested experimentally in comparison with a conventional strategy. The efficacy these CPS on pest and pathogen control was reported in Chapter 5 and, among agronomic performances, only the yields and the quantity of pesticides residues found in harvested lettuces will be presented and discussed here. The chapter rather focuses on the economic and environmental performances of the CPS. The CPS were implemented in three locations during two winters and the crop performances were assessed in each case, as well as globally, to analyze the costs and benefits of pesticide reduction.

6.2 Material and Methods

6.2.1 Designing of crop protection strategies

Three CPS were designed by a group of experts of lettuce production in Southeast France. A conventional CPS, representative of current practices in protected winter lettuce crops, was elaborated from the synthesis of four farmer's practices. A second CPS was built to maintain economic and agronomic standards, while moderately reducing pesticide applications by introducing alternative techniques (intermediate CPS). A third CPS, called low-input, was designed to sharply reduced inputs and was intended to explore the technical boundaries of pesticide reduction. A substitution of pesticides by alternative techniques was proposed, to a various extent, and the techniques were applied from fixed or contingent decision rules to suit local constraints.

6.2.2 Experimental design

The three CPS (conventional, intermediate and low-input CPS) were implemented in Batavia production under high plastic tunnels, in 3 locations in southeast France during winters 2012-2013 (W1) and 2013-2014 (W2). A total of 18 experimental plots, with a surface area between 100 and 250m^2, were observed (3 CPS x 3 locations x 2 years). Two locations were conventional farms located at Candillargues (L1) (43 ° 62'N ; 4 ° 06'E ; 3 m elevation) and at Villelongue de la Salanque (L2) (42 ° 73'N ; 2 ° 98'E ; 6 m elevation) in the region Languedoc Roussillon, France. The third location (L3) was the INRA experimental station located at Avignon (43 ° 91'N ; 4 ° 87'E ; 31 m elevation) in the region Provence Alpes Cote d'Azur (PACA), France. Crop cycle duration was very similar among experiments, between 70 and 84 days (cf. Table 5.1). The various combinations of location and years are thereafter typed as L1-W1, L1-W2, L2-W1, L2-W2, L3-W1 and L3-W2.

6.2.3 Crop protection strategies

Several techniques to manage lettuce pests and diseases, reviewed in Barrière et al. (2014) have been used in the conventional, intermediate and low-input CPS and are described below. The targets of each technique are given in Table 6.1.

Pesticide applications

All pesticides were applied with a spray boom. Fungicides were applied following a calendar-based program while insecticides were applied following regional alerts (conventional CPS) or when the abundance of aphids had reached species-dependent thresholds. The count was made on 40 lettuces per plot, randomly selected every week. The threshold for the specie N. ribisnigri was 2% and 10% of lettuce plants infested in plots under intermediate CPS and low-input CPS respectively. For other aphid species, it was 5% and 15% respectively. For each CPS, the set of pesticide applications is given in Table 6.2 (for a more accurate description of pesticides applied see Annexe 2). The Treatment Frequency Index (TFI) (Gravesen 2003 ; Champeaux 2006) was

used to assess the amount of pesticide applied in each CPS. TFI accounts for the number of molecules, the number of treatments and the dose per surface, as follow :

$$TFI = \sum \frac{\text{Applied rate} \times \text{Area treated}}{\text{Authorized minimal dose} \times \text{Plot area}}$$

Biological control

In all the CPS, one application on the soil surface of the fungus *Coniothyrium minitans* (Contans®) at a rate of 2 kg.ha^{-1} was done before the first lettuce crop (in October). A second application was carried out before planting in the intermediate- and low-input CPS. Additionally, for the low-input CPS, the biocontrol fungus *Trichoderma harzianum* (strain T22 – Trianum P®) was applied twice on the balls, at the cotyledon stage (at 1.5 g.m^2) and just prior to planting (at 1 kg per 8500 plants).

Mixtures of aphid parasitoids (Basilprotect®) including *Aphidius colemani*, *Aphidius ervi*, *Aphidius matricariae*, *Aphelinus abdominalis*, *Aphedrus cerasicola* and *Praon volucre* were preventively introduced at the center of each plot cultivated under intermediate CPS and low-input CPS at a rate of 1.2 individuals per m^2 every two weeks.

Irrigation

Except for the plot cultivated under low-input CPS in L3, all the plots were sprinkler-irrigated throughout crop growth. Just after planting, water was applied to field capacity. The moisture content was maintained in the balls containing the plantlets by short daily irrigations until the roots started to grow into the soil. After this early growth stage, drip irrigation was used under low-input CPS in L3 only. Irrigation was triggered according to the soil water potentials measured by 6 Watermark®sensors placed at 3 positions and 2 depths (15 cm and 35 cm), following the current guidelines in SE France (Bressoud 1998). At the other sites and CPS, sprinkler irrigation was triggered once every two or three weeks, according to ETP. Towards the end of cropping cycle, short sprinkler irrigations were triggered when the temperature exceeded 30 ° C, to allow rapid cooling of the lettuces.

Fertilization

A few days before planting, about fifteen 30 cm deep soil samples were randomly collected in each plot. Soil nitrate content was measured in each plot with a Nitrachek reflectometer. Nitrogen fertilization was calculated to assess the fertilizer requirements. In the conventional CPS and

Crop protection techniques	Main pests and pathogens of winter lettuce crops					
	B. lactucae	*S. sclerotiorum*	*S. minor*	*B. cinerea*	*R. solani*	Aphid species
Fungicides	x	x	x	x	x	
Insecticides						x
Biological control agents		x	x	x	x	x
Resistance inducers	x					
Drip irrigation	x	x	x	x		
Reduced N- fertilization		x	x	x		
Genotype	x	x				
Planting density		x	x	x	x	
Infected plant management	x	x	x	x	x	x

TABLE 6.1: Pest and pathogen targets of crop protection techniques used in the three CPS.

CPS	Fungicides	Resistance inducers	Insecticides
Conventional	8 days (± 2 days) after planting: I + Si 20 days (± 2 days) after planting: Sy + Sw 30 days (± 2 days) after planting: I + Ro 40 days (± 2 days) after planting: Sy	/	One insecticide, Mo, Pi or Su was applied 7 seven days after aphid observation in the area of production.
Intermediate	10 days (± 2 days) after planting: I + Si 32 days (± 2 days) after planting: Sy + Sw	22 and 44 days (± 2 days) after planting : Lb	One insecticide, Mo, Pi or Su was triggered when the abundance of aphids had reached species-dependent thresholds
Low-input	10 days (± 2 days) after planting: I 20 days (± 2 days) after planting: Si 32 days (± 2 days) after planting: Sy	22 and 44 days (± 2 days) after planting : Lb	One insecticide, Mo, Pi or Su was triggered when the abundance of aphids had reached species-dependent thresholds

TABLE 6.2: Pesticide applications performed in each experimental plot cultivated under conventional, intermediate or low-input CPS. Letters in parentheses give the commercial names of pesticides. I : Infinito (Bayer) ; Ro : Rovral (BASF Agro) ; Si : Signum (BASF Agro) ; Sw : Switch (Syngenta Agro) ; Sy : Sygan (Dupont Solutions) ; Mo : movento (Bayer) ; Pi : Pirimor G (Syngenta Agro) ; Su : Suprême (Certis Europe) ; Lb : LBG 01F34 (de Sangosse).

intermediate CPS, fertilization followed the farmer's practices, consisting in the adjustement of soil N to 100-120 kg NO_3 -N.ha^{-1}, with mixed N-$P_2O_5K_2O$ fertilizers (either 10-20-20 or 5-7-9 depending on the site). If nitrate content exceeded 120 kg.ha^{-1} prior to planting, no fertilizer was applied.

For plots cultivated under low-input CPS, nitrate fertilization was reduced and split. Before planting, soil nitrate-N content was adjusted to 40kg.ha^{-1}. At the 16^{th} leaf stage, a new soil sample was collected and analyzed following the same procedure, and, using this updated measurement, the soil nitrate-N content was adjusted to 60 kg.ha^{-1}. Due to significant N mineralization during early growth, the soil nitrate stock at this stage was usually not exhausted, resulting in low fertilizer applications. As a result, the total application of N fertilizers in the plots cultivated under low-input CPS was much lower than in the two other CPS.

Genotype

A different cultivar was used in each CPS. Cv Notilia (Clause), used in the conventional CPS, is a fast-growing variety and has almost a complete range of resistance to *B. lactucae* (Bl 1-28, 30, 31). Cv Ostralie (Rijk Zwaan), was used in the intermediate CPS. This cultivar grows slightly more slowly, has a complete range of resistance to *B. lactucae* (Bl : 1-31) and has a semi-upright habit. In the low-input CPS, Lasydo (Syngenta seed), a fast-growing variety with an almost complete range of resistance to *B. lactucae* (Bl 1-28, 30, 31) and a low susceptibility to *S. sclerotiorum* (cf. Chapter 3), was used.

Planting density

Lettuces were planted in parallel rows, on micro- and macro- perforated black plastic mulch (Optimac®). The planting density in low-input CPS was reduced from 14.25 to 12.75 plants per square meter.

Infected plant management

In low-input CPS, any lettuce showing pathogen damage rendering the plant unmarketable was carefully removed from the plot in order to prevent inoculum dissemination.

Lettuce gross and marketable yields

At harvest, 60 lettuces for each CPS were randomly sampled and used to assess production performance of each CPS. The percentage of marketable lettuces (%ML), the average lettuce fresh weight (LW, g), the marketable weight (after the removal of unmarketable basal leaves, MLW, g), and the percentage of unmarketable basal leaves were measured. The gross yield (t fw.ha^{-1}) was calculated as :

$$\text{Gross yield} = \frac{LW \times \text{planting density}}{100}$$

The marketable yield (t fw.ha^{-1}) was calculated as :

$$\text{Marketable yield} = \frac{MLW \times (\%ML) \times \text{planting density}}{100}$$

Number of pesticide residues detected at harvest

Five additional plants were randomly sampled at harvest and checked for the presence of 287 pesticide residues including dithiocarbamates and phosphorous acid by mass or UV spectrometry. A score for pesticide residues was given as the sum of the individual compounds identified in each experimental plot.

Assessment of economic performances

The production costs depended on the practices and amount of inputs used in each CPS. Inputs, amortization and labor costs were collected from farmers of the L1 and L2 production sites or from input suppliers. For L3, the experimental station, we used the mean values of L1 and L2 when prices data missed or were not representatives of current production sites. Prices reported are excluding taxes and correspond to those applied during the experiments. Total costs (€.ha^{-1}) per plot are the sum of input, amortization and labor costs. For each CPS, the profitability limit, which is the balance between production costs and sale returns, has been calculated using a sale price of 0.8 €.kg^{-1}.

6.2.4 Assessment of environmental performances

Environmental Impact Quotient

The environmental impact of pesticide program was assessed in each CPS by calculating its Environmental Impact Quotient (EIQ) (Kovach et al. 1992). This quotient is based on the dose, the persistence and the toxicity, for farm workers, consumers and ecological components (fishes, birds, bees and beneficial arthropods), of active ingredients applied. Partial EIQ were also calculated for each type of pesticide applied (fungicides, insecticides, resistance inducers).

Macro-arthropods abundance and diversity

In order to investigate the impact of pesticide reduction on epigeic macro-arthropods, Barber pitfall traps were laid during W1 and W2. Since the composition of macrofauna community mainly relies on location, the diversity and the abundance of arthropods were investigated by location and only in L1 and L3. In each experimental plots, six pitfall traps were laid at planting between the crop and the tunnel wall (3 traps in each side). Two and four weeks after planting, the traps were collected and replaced. The last traps were retrieved about 45 days after planting. In total, 216 pitfall traps were collected (6 traps x 3 dates x 3 CPS x 2 locations x 2 winters).

Only the arthropod macrofauna was considered (thus excluding Collembola, Aphididae and Acari). Depending on their abundance, the taxonomic rank of arthropods trapped during experiments was determined mainly at the phylum level (for myriapods), at the order level (Hymenoptera, Diptera, Orthoptera, Homoptera, Coleoptera, Dermaptera and Opiliones) and at the family level for spiders (Gnaphosidae, Lycosiae, Linyphiidae). The abundance of epigeic arthropods was defined as the average number of individuals per pitfall trap and the Shannon-Wiener index (H') (Shannon and Weaver 1949) was calculated using the taxonomic rank described above for each experimental plot of L1 and L3 as :

$$H' = \sum_{i=1}^{S} Pi \times ln \times Pi$$

Where Pi is the proportion of individuals belonging to the i^{th} taxon and S is the total number of taxons.

6.2.5 Data analysis

Considering the small size of the sample (18 values per variable), non-parametric Kruskal-Wallis rank sum tests (with a significance threshold of 0.05) were used to analyze separately the effect of CPS, locations or winters on gross yield, marketable yields, percentage of marketable lettuces, number of pesticide residues, EIQ and total costs. The differences of arthropod abundance and of Shannon-Wiener index (H') among CPS were tested, in L1 and L3, also using Kruskal-Wallis rank sum tests. Then, rank-based multiple comparison test (De Mendiburu 2009) were used when significant differences among CPS, location or winter were found. All statistical analyzes were performed with R (R-Core-Team 2013).

6.3 Results

6.3.1 Effect of CPS on gross and marketable yields

Average gross yields were slightly lower in the intermediate and low-input CPS as compared to the conventional one, but the differences were not statistically significant (Table 6.3). These differences might have been due to the choice of a slow growing genotype in the intermediate CPS or to the reduced planting density in the low-input CPS. Despite a high variability between plots, there was also neither effect of location (P= 0.849) nor winter (P= 0.566). Abiotic damage (frost and tip burn) affected some of the plots, to a varous extent, depending on the cultivar, and hence the CPS. Significant differences in aphid abundance were also observed in the low-input CPS (sata not shown)but they had less impact on the commercialization than abiotic damage. As a result, a high variability in the percentage of marketable lettuces was observed in every CPS, but no significant difference between CPS was observed (Table 6.3). No differences were found among locations ($P_{\text{marketable yield}}$= 0.7961 ; $P_{\%\text{ marketable lettuces}}$= 0.3033) or winters ($P_{\text{marketable yield}}$= 0.7573 ; $P_{\%\text{ marketable lettuces}}$= 0.6261). However, the number of pesticide residues found in marketed plants was significantly higher in the intermediate strategy (P= 0.01902) than in the two others.

6.3.2 CPS production costs and profitability

The production costs were significantly different among CPS (P= 0.001675), while no significant difference was observed among locations (P= 0.3594) or winters (P= 0.7573). Total production costs were 30 984 €. ha^{-1}, 35 105 €. ha^{-1} and 33 986 €. ha^{-1} in the conventional, intermediate and low-input CPS, respectively (Figure 6.1). Pesticide costs were reduced by 27% in the intermediate CPS and by 49% in the low-input CPS. However, they represented only 1.5% of the total costs in the conventional CPS, and even less in the other CPS. Labor costs accounted for 32-33% of the total costs in all CPS, but the absolute amount was higher in the intermediate and low-input CPS, mainly due to additional pest surveillance requirements. Biological control

CPS	Gross yield ($t.ha^{-1}$)	Marketable lettuces (%)	Marketable yield ($t.ha^{-1}$)	Residue Number
Conventional	51.5 (±15.2)	71.4 (±28.5)	36.3 (±6.7)	3.50 (±1.1) b
Intermediate	47.9 (±13.7)	82.9 (±24.2)	39.9 (±6.3)	4.83 (±0.8) a
Low-input	45.8 (±11.0)	78.3 (±10.1)	36.0 (±10.7)	2.50 (±1.4) b
P-value	0.9599	0.3212	0.7378	< 0.05

TABLE 6.3: Yield and number of pesticides residues measured in the three crop protection strategies (means ± standard deviation of 2 winters x 3 locations for each CPS). Different letters in a column indicate significant differences according to a Krukal-Wallis multiple comparison test at 5% confidence level.

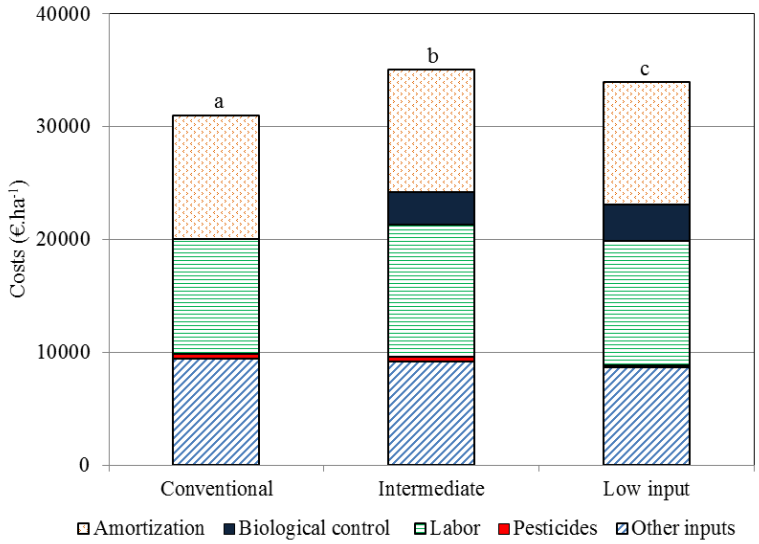

FIGURE 6.1: Production costs of the three crop protection strategies, distributed over different items of expenditure. Different letters above the bars indicate significant differences according to a Krukal-Wallis multiple comparison test at 5% confidence level.

accounted for almost all the additional costs in the intermediate and low-input CPS. They represented 0.1, 8.2 and 9.3% of the total costs in the conventional, intermediate and low-input CPS, respectively. Despite the wide range of alternative techniques used in the low-input CPS, its total cost appeared lower than that of the intermediate CPS. This can be explained by a decrease of labor cost at harvest due to the lower planting density, and by decreased costs of seedlings and fertilizers.

As a consequence of higher production costs, the profitability limit, given as the combination of lettuce weight and number of marketable lettuces per hectare necessary to reach a zero profit, was slightly in alternative CPS (Figure 6.2). Despite the lower planting density in the low-input CPS, the profitability limit was lower in this CPS. In fact, the planting density did not affect the gross yield in the low-input CPS, while the production cost was higher for the intermediate CPS. For similar yields, the increase of lettuce selling price by 13% in the intermediate CPS and by 10% in the low-input CPS would allow a similar profitability as that of the conventional CPS.

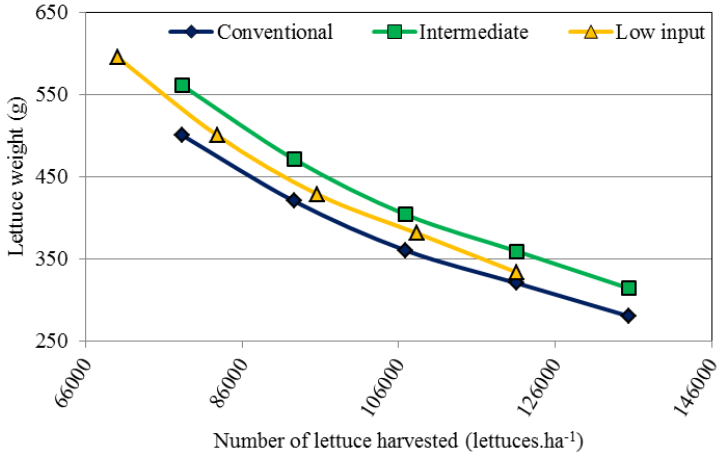

FIGURE 6.2: Combination of marketable yield components (lettuce weight and number of marketable lettuces per hectare) determining the limit of profitability of each CPS for a sale price of 0.8€.kg^{-1}.

6.3.3 Environmental performances of CPS

Environmental impact quotient

Pesticides were reduced by 31.8% in the intermediate CPS and by 47.7% in the low-input CPS. EIQ values were different among CPS (P= 0.0004648) but not among locations (P= 0.9551) nor between winters (P= 0.7898) (Table 6.4). The average total EIQ were 207, 159 and 95 for the conventional, intermediate and low-input CPS, respectively, and as expected decreased fallowing TFI. Fungicides accounted for 90% of the total EIQ value. In the intermediate and low-input CPS, resistance inducers accounted for 9 and 16% of EIQ, respectively.

Diversity and abundance of epigeic arthropods

The quantity and the nature of the epigeic arthropods were different between locations. The total abundance of the epigeic macrofauna in L1 was significantly higher in the low-input and intermediate CPS compared to the conventional one. Significant differences were as well observed for some taxons with higher number of spiders (Linyphiidae) and Coleoptera and a lower number of Myriapods both in the intermediate and low-input CPS than in the conventional one (Table 6.5). Hymenoptera (mainly ants) were also more numerous in the low-input CPS than in the other CPS. No significant difference was observed between CPS for diversity (H').

In L3, the total abundance of arthropods observed was significantly higher in the low-input CPS compared to the conventional CPS. Some taxons showed as well significant differences

CPS	TFI	EIQ fungicide	EIQ insecticide	EIQ resistance inducers	EIQ total
Conventional	8.8	203.5	3.4	0.0	206.9 a
Intermediate	6.0	141.5	2.8	14.8	159.1 b
Low-input	4.6	79.0	0.6	14.8	94.4 c

TABLE 6.4: Treatment Frequency Index (TFI) and Environmental Impact Quotient (EIQ) values for the different types of chemicals applied (fungicide, insecticide and resistance inducers) in the three crop protection strategies (mean of 3 locations x 2 winters). Different letters in a column indicate significant differences according to a Krukal-Wallis multiple comparison test at 5% confidence level.

CPS	Abundance	H'	Linyphiidae (spider)	Myriapods	Coleoptera	Hymenoptera
Conventional	25.2 b	1.92	2.44 c	6.05 a	2.15 b	6.05 a
Intermediate	29.2 a	1.93	4.65 a	3.65 b	3.60 a	3.65 b
Low-input	29.7 a	1.85	3.12 b	3.05 b	3.14 a	3.02 b
P- value	0.04	Ns	P <0.001	P <0.001	P= 0.01	P <0.001

TABLE 6.5: Characteristics of arthropod communities (average abundances and Shannon-Wiener index (H') for two years) in each CPS in L1. Different letters in a column indicate significant differences according to a Krukal-Wallis multiple comparison test at 5% confidence level.

CPS	Abundance	H'	Lycosidae (spider)	Diptera	Homoptera
Conventional	18.9 b	1.58 b	1.38 a	4.48 b	0.71 a
Intermediate	20.4 b	1.84 ab	0.70 ab	5.81 b	0.37 b
Low-input	22.9 a	1.79 a	0.27 b	8.21 a	0.20 b
P- value	P= 0.03	P= 0.04	P <0.01	P= 0.01	P= 0.03

TABLE 6.6: Characteristics of arthropod communities (average abundances and Shannon-Wiener index (H') for two years) in each CPS in L3. Different letters in a column indicate significant differences according to a Krukal-Wallis multiple comparison test at 5% confidence level.

between CPS with higher number of spiders (Lycosidae) and Homoptera (mainly Ciccadellidae) and a lower number of Diptera in the conventional CPS compared to the low-input CPS (Table 6.6).

When looking at the variation of abundance along time (between the first and the last sampling date), for both locations, either larger increases (in W1) or smaller decreases (in W2) in the low-input CPS were always observed (Figure 6.3). The sign of this evolution (decrease or increase) is likely to depend on the climatic conditions at the start of the experiment. During W1, the less pesticides were applied, the more arthropod abundance had increased. In the same way, during W2, the more pesticides were applied, the more abundance had decreased.

6.4 Discussion

To allow the adoption of alternative techniques by farmers and to improve the sustainability of production systems, agricultural research must demonstrate the feasibility and the economic and environmental impacts of reducing pesticides. Our question was to investigate the risk and the added value of crop protection strategies with significant pesticide reductions. To our knowledge, this is the first study with this aim on lettuce. The alternative protection strategies tested have maintained the gross yield (even in the low-input CPS that reduced planting density) and the percentage of marketable lettuces (which can be affected by pest and pathogen damage). These results demonstrate the possibility of pesticide reduction in conventional lettuce crops using combinations of alternative techniques. In the low-input CPS, pesticide reduction has reached 48% which is practically the objective of the French governmental Plan Ecophyto (2008). However, despite similar yields, the increase of production costs in the intermediate and the low-input CPS resulted in an decrease of profitability in those CPS compared to the conventional CPS. Considering the low share of pesticide in the total production costs of conventional strategies, reducing the costs of chemical control did not compensate for additional expenses in the intermediate and low-input CPS. This additional cost was, however, mainly due to biological control products, especially the parasitoids, introduced for aphid management. This suggests that the use of a less expensive alternative could help to balance the performance of alternative and conventional strategies.

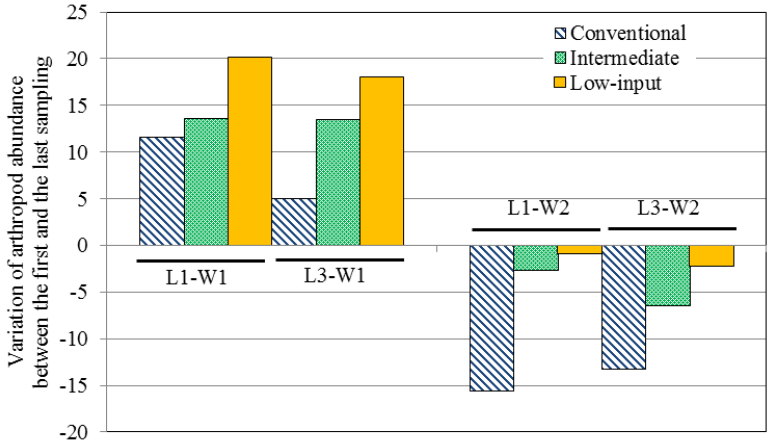

FIGURE 6.3: Net evolution of the mean abundance of macro-arthropods between the last and first sampling date for each site and winter.

Moreover, the introduction of parasitoids and several other alternative techniques used in this study are not currently adopted by farmers. A wider adoption of these alternative techniques could lead in some cases to economies of scale. Accordingly, the costs of alternative strategies might have been slightly overestimated.

An increase of the price of lettuce grown with less pesticide could offset the differences of profitability between CPS. The low-input CPS, which has reduced by half the use of pesticides, would require only a 10% price increase. Consumers are generally concerned about health and environmental hazards related to pesticides : 67% of consumers are willing to pay 10% more expensive fruits and vegetables grown under more environmentally friendly practices (Boccaletti and Nardella 2000). Therefore, the creation of an additional market segmentation to support the additional costs related to pesticide reduction in lettuce crops might be considered. Nonetheless, while alternative strategies appeared to have less adverse impacts on the environment, it seems that the use of less pesticides do not guarantee the marketing of a product with fewer pesticide residues, since the number of pesticide residues detected at harvest was higher in the intermediate CPS and not significantly lower in the low-input CPS. This drawback should be considered to improve or to design new alternative strategies for pest and pathogen management. In addition to the timing of chemical treatments, a number of factors affecting the number of residues of pesticides detected in plants have already been identified, including the properties of the applied active ingredients, the plant features (variety, growth stage) and the environmental conditions (temperature, humidity) (Edwards 1975 ; Fantke et al. 2014).

Furthermore, although it is not estimated in the present study, the use of pesticides has a higher cost than just their purchasing cost. In the United States, the cost of pesticide impact on public health, on beneficial organisms such as predators and pollinators, on ground and surface water, on fishery, etc. has been estimated to 9645 million dollars per year (Pimentel 2005). In the present study, the EIQ value gave an indicator of the possible gains of pesticide reduction in lettuce crops. The EIQ have been reduced by 23 and 54% in the intermediate and low-input CPS, respectively, mainly because of fungicide reduction. In comparison, Clark et al. (1998) have found an EIQ reduction of 37 to 41% in low-input tomato cropping systems compared to conventional cropping systems.

An overall reduced abundance of arthropods was observed in the conventional crop protection strategies. It is difficult to specifically connect this phenomenon to pesticides since other practices can impact the arthropod community such as fertilization, tillage and crop rotation (Edwards 1984 ; Thorbek and Bilde 2004). Here, crop rotation and tillage were identical in the three CPS but N mineral fertilization was reduced in the lower input CPS. However, the variation of arthropods abundance between the first and the last sampling dates suggests that successive pesticide

applications could be at least partly responsible for the decline in arthropod abundance.

The abundances of some taxons including Linyphiidae, Coleoptera, Hymenoptera, and Diptera, were higher in the plots cultivated under alternative strategies than under conventional strategy. Not all of these taxons can be considered as predators but Linyphiidae spiders are, and some Coleoptera (Carabidae and Staphylinidae) as well. On the other hand, Lycosidae, Myriapods and Homoptera had lower abundances in the plots cultivated under alternative strategies than under conventional strategy. This balancing is not surprising since the decline of a taxon is generally offset by the development of another one less susceptible to the disturbance and with the same ecological niche (Edwards 1984).

Since the vast majority of pesticides used in this study were fungicides, it is likely that they had sublethal rather than lethal effect on arthropods. Fungicides may have affected the physiology (development, fertility, longevity) and the behavior of arthropods (mobility, search of prey / host) (Desneux et al. 2007). In general, only the direct lethal effect of pesticides are taken into account when assessing the impact of active ingredients on non-target organisms (Desneux et al. 2007). Therefore, measuring the abundance and the diversity of epigeic arthropods was complementary to the EIQ calculation to define the environmental performances of CPS.

6.5 Conclusion

In this study, we have shown the efficacy of the crop protection strategies reducing pesticide use and their interest for the sustainability of lettuce production systems. Considering the variability of production situations, further studies are needed to confirm these results. The adoption of alternative CPS might relies on a reduction of biological control cost or on the creation of additional market segmentation with a slightly increase of selling prices.

6.6 Acknowledgements

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Quatrième partie

Discussion générale

Une approche analytique et une approche intégrative ont été combinées dans cette étude afin d'examiner la faisabilité, ainsi que les coûts et les bénéfices économiques et environnementaux, d'une réduction de pesticides en culture de laitue. Ces deux approches ont apporté des informations substantielles, pour la gestion de certains pathogènes et ravageurs de la laitue, qui seront rappelées et discutées en premier lieu. Une attention particulière sera portée à l'efficacité et à l'intégration des pratiques culturales ayant un effet sur plusieurs bio-agresseurs telle que la fertilisation azotée ou encore l'espacement des laitues. Ensuite, la discussion s'orientera sur les avantages et les limites de la démarche scientifique articulant approche analytique et intégrative.

Gestion des bio-agresseurs de la laitue : apports de la thèse et perspectives d'amélioration des stratégies de protection alternatives

Gestion des pathogènes responsables de la pourriture du collet ('Basal rot')

La pourriture du collet est un symptôme qui peut être causé par plusieurs pathogènes de la laitue dont *S. sclerotiorum*, *S. minor*, *B. cinerea* et *R. solani* (Van Beneden et al. 2009). Dans le chapitre 3, l'effet du génotype et des teneurs en sucres solubles sur la sensibilité des laitues à deux des principaux responsables de la pourriture du collet, *B. cinerea* et *S. sclerotiorum*, a été analysé. L'étude a révélé que, pour un nombre réduit d'accessions, la variabilité de réponse à *B. cinerea* et à *S. sclerotiorum*, non liée à l'architecture des plantes, était relativement importante pour les deux pathogènes (allant d'un facteur de 1 à 4 pour *S. sclerotiorum* et de 1 à 7 pour *B. cinerea*). Le génotype a rendu compte d'une partie significative de la variabilité observée dans chaque expérience, tandis que les variations de sensibilité observées entre les expérimentations ont pu être reliées aux teneurs en sucres solubles (fructose et saccharose). La teneur en fructose était positivement corrélée à la vitesse de développement de *B. cinerea* tandis que la teneur en saccharose était négativement corrélée à la vitesse de développement des deux pathogènes. Nous avons conclu que la gestion de ces deux pathogènes pourrait être améliorée en utilisant des variétés de laitues présentant, indépendamment de leur architecture, un certain niveau de résistance, et en mettant les plantes dans des conditions de croissance favorables à l'expression de faibles ratios fructose : saccharose.

Dans l'approche intégrative, les stratégies alternatives de gestion de la pourriture du collet combinant lutte biologique et architecture de plante semi-érigée pour la stratégie intermédiaire et lutte biologique, variété peu sensible à *S. sclerotiorum*, réduction de la fertilisation azotée, de la densité de plantation et retrait des plantes infectées pour la stratégie bas-intrant ont permis de réduire l'usage des pesticides d'un tiers et deux tiers respectivement par rapport à la stratégie conventionnelle. L'approche intégrative a mis en évidence l'effet prépondérant du climat et du microclimat sur l'incidence des symptômes. En effet, les différences significatives observées entre les sites expérimentaux et les différences de poids observés entre laitues saines et laitues infectées révèlent que la présence d'inoculum primaire et de conditions favorables au développement des pathogènes responsables de la pourriture du collet ont été des déterminants majeurs pour le développement de la maladie.

Lors de la conception des stratégies alternatives, nous avions connaissance des résultats du criblage préliminaire des accessions des laitues pour leur sensibilité à *B. cinerea* et *S. sclerotiorum* (Annexe 1). Nous avons donc utilisé pour la stratégie bas-intrant, une accession qui présentait une faible sensibilité à *S. sclerotiorum* (i.e. Lasydo, Syngenta). La relation entre les teneurs en sucres solubles et la sensibilité n'avait cependant pas encore été mise en évidence. Une des perspectives majeures pour l'amélioration de la gestion intégrée de la pourriture du collet est de mieux définir les conditions de croissance adéquates pour l'expression des ratios de sucres solubles défavorables au développement des pathogènes (fertilisation, intensité lumineuse, température, etc.) et de voir

si celles-ci sont compatibles avec les conditions climatiques et microclimatiques qui restreignent les infections.

Gestion de *B. lactucae*, l'agent du mildiou des composées

Les techniques de gestion de *B. lactucae* intégrées à la fois au sein de la stratégie intermédiaire et de la stratégie bas-intrants ont consisté en des applications de stimulateurs de défense (phosphites), la plantation de variétés résistantes et l'utilisation d'un dispositif d'irrigation goutte à goutte dans L3. Cette stratégie alternative s'est révélée efficace pour gérer le mildiou des composées mais n'a permis de réduire l'IFT que de 21.5%. Les stratégies alternatives, testées dans l'expérimentation systémique, reposent encore sur des calendriers de traitements préétablis alors que le développement de *B. lactucae* est très dépendant du climat. La sporulation nécessite, par exemple, une forte humidité relative et des températures comprises entre 5 et 15 ° C (Nordskog et al. 2007). Une réduction plus importante aurait pu être atteinte si les applications de pesticides et de stimulateurs de défenses avaient été réalisées en fonction du risque épidémiologique. Des modèles de prévision du développement de *B. lactucae* sur les cultures de laitue, basés sur les conditions climatiques ont été développés (Kushalappa 2001 ; Hovius et al. 2007). Cependant, l'utilisation de ces modèles, développés en Amérique du Nord, nécessite leur adaptation pour les cultures sous abris du Sud de la France.

Gestion des pucerons

Palumbo et al. (2009) ont suggéré que la lutte biologique ne permettait pas un contrôle suffisamment rapide des populations de pucerons sur laitue pour prévenir les pertes économiques. Grâce à l'approche intégrative, nous avons constaté que les stratégies de gestion alternatives des pucerons, intégrant lutte biologique par introduction préventive de parasitoïdes et seuils de traitement chimique, pouvaient être aussi efficaces que la stratégie conventionnelle pour certaines espèces de puceron, telles que *M. persicae*, attaquant la laitue en hiver sous abri. Cependant, pour l'espèce *N. ribisnigri*, qui se développe préférentiellement au cœur des laitues (Liu 2004), la lutte biologique n'a pas permis de maintenir, dans les conditions d'un essai, la population en dessous des seuils de traitements chimiques. Par ailleurs, le coût des agents de lutte biologique appliqués (mélange d'espèces d'*Aphidius*) a compté pour l'essentiel du surcoût de production des systèmes alternatifs par rapport au système de production conventionnel. L'approche analytique, développée dans le chapitre 4, nous a permis de comprendre les raisons de cet échec. Les parasitoïdes en fin de culture ont eu des difficultés à rencontrer leurs hôtes. L'efficacité contrastée des parasitoïdes dans les deux approches ainsi que leur coût a révélé la nécessité de reconsidérer les stratégies alternatives de gestion des pucerons, ou toutefois de *N. ribisnigri*. La première option pour améliorer ces stratégies pourrait être de diminuer les seuils de traitements chimiques. Cependant les seuils utilisés pour l'espèce *N. ribisnigri* étaient déjà très bas. Le seuil de traitements chimiques (2% des plantes atteintes) de la stratégie intermédiaire était bien en dessous du seuil de tolérance des usines de $4^{ème}$ gamme pour la présence de corps étrangers. De ce fait, les traitements ont été effectués dès la première observation de *N. ribisnigri* et n'ont pas permis de réduire l'usage des insecticides. De plus les applications d'insecticides, dans les deux stratégies alternatives, ont ralenti mais n'ont pas stoppé le développement de la population de pucerons, conduisant à des pertes de rendement faibles, mais significativement supérieures par rapport à la stratégie conventionnelle. Le manque d'efficacité curative des insecticides constitue en conséquence un frein majeur pour l'utilisation des seuils de traitement chimiques.

Comme souligné dans le chapitre 4, la seconde option pour l'amélioration des stratégies alternatives, est d'intégrer de nouveaux moyens de gestion des pucerons. Une légère augmentation ou diminution de la fertilisation azotée ainsi que la limitation de la dispersion des populations de pucerons, par exemple par la modification de l'espacement entre les laitues, pourraient être envisagées. L'introduction de nouveaux agents de lutte biologique, ou une modification de l'environnement des parcelles favorisant la présence de prédateurs des pucerons restent également des options qui pourraient être intégrées à l'avenir dans les itinéraires techniques.

Techniques ayant des effets sur plusieurs bio-agresseurs

Les pesticides ont généralement un effet biocide, ou du moins un impact négatif, sur plusieurs pathogènes ou ravageurs. La réduction des pesticides peut, en conséquence, entraîner l'émergence de nouveaux problèmes phytosanitaires (Brismontier et al. 2009) si d'autres techniques à large spectre ne sont pas utilisées. En combinant les deux approches, nous avons étudié l'effet des techniques alternatives à large spectre telles que la réduction de la fertilisation azotée et l'augmentation de l'espacement des laitues sur les bio-agresseurs cibles et non-cibles.

Fertilisation azotée

La fertilisation azotée détermine partiellement la sensibilité des plantes aux bio-agresseurs car elle affecte à la fois leur croissance, leurs mécanismes de résistances et le développement des bio-agresseurs via la modification de leurs ressources nutritives (Dordas 2008 ; Altieri and Nicholls 2003 ; Walters and Bingham 2007). Avant cette étude, nous savions que les hauts niveaux de fertilisation azotée pouvaient augmenter la sévérité des attaques de limaces (Deroceras sp.)(Pakarinen et al. 1990), ainsi que celles de *B. cinerea* et *S. sclerotiorum* (Lecompte et al. 2013). Cependant, nous ne savions pas dans quelle mesure la réduction de la fertilisation azotée pouvait impacter les autres bio-agresseurs de la laitue. L'approche intégrative nous a permis de vérifier que cette réduction, intégrée à la stratégie bas-intrant, n'entraînait pas de recrudescence de bio-agresseurs non-cibles. En outre, l'approche analytique a montré qu'une réduction de la fertilisation azotée pouvait également défavoriser la croissance des populations de *N. ribisnigri*.

Optimisation de l'espacement entre les laitues

La combinaison des approches analytique et intégrative a révélé que l'espacement entre les laitues avait un effet sur plusieurs bio-agresseurs. Lorsque l'espacement entre les laitues est important, il limite le développement de conditions favorables aux pathogènes responsables de la pourriture du collet. Cet effet avait déjà été mis en évidence pour *S. minor* sur cultures d'arachides (Maas et al. 2006). Par ailleurs, si l'espacement entre les laitues est suffisant pour que celles-ci ne soient pas en contact direct, il est probable qu'il ralentisse la propagation des pucerons dans la culture. L'espacement entre les laitues dépend à la fois du volume de la variété plantée, de la densité de plantation et de la géométrie de plantation (ligne ou quinconce). Pour garantir les rendements, tout en optimisant l'espacement entre les laitues, nous suggérons de planter des laitues compactes (lourdes mais peu volumineuses) et en quinconce, car cette structure limite les espaces vides et retarde le moment où les laitues entrent en contact. Une légère réduction de la densité de plantation peut également être utilisée car nous avons montré qu'elle n'induisait pas de pertes de rendement lorsque les laitues étaient vendues au poids.

Avantages et limites de la démarche scientifique

Avant cette étude, peu d'informations étaient disponibles sur la gestion intégrée des bio-agresseurs de la laitue. Des démarches intégratives des gestion d'un bio-agresseur (Fagan et al. 2010) ou d'une partie des bio-agresseurs de la laitue (Collange et al. 2014 ; Navarrete et al. 2010) ont précédemment été réalisées. Cependant, à notre connaissance, la présente étude est la première visant à contrôler l'intégralité des bio-agresseurs pouvant menacer la culture de laitue d'hiver cultivée sous tunnel.

Les deux approches, analytique et intégrative, utilisées ici, possèdent des avantages et des inconvénients pour le développement de solutions alternatives de gestion des bio-agresseurs.

L'approche analytique a permis de caractériser l'effet des techniques étudiées sur les bio-agresseurs cibles et de proposer des ébauches de techniques alternatives nouvelles à intégrer dans les stratégies de gestion des bio-agresseurs. Elle a également apporté des informations pour une

amélioration des techniques déjà utilisées. Cette approche a également permis de comprendre certains résultats observés dans les essais systémiques, telle que l'inefficacité du parasitisme de *N. ribisnigri* par les *Aphidius*. Cependant ces essais ne sont pas destinés à étudier la faisabilité et les conséquences d'une réduction des pesticides.

L'approche intégrative, quant à elle, a permis de confronter les stratégies conçues aux objectifs de production et aux exigences de la filière et de vérifier la compatibilité des techniques misent en place et l'efficacité des combinaisons. En plus de leurs impacts positifs sur l'environnement, les stratégies alternatives ont été suffisamment efficaces pour obtenir une qualité visuelle et des rendements équivalents à la stratégie conventionnelle. Telles quelles, ces stratégies pourraient être adoptées par les producteurs si les laitues produites étaient valorisées via la création d'une segmentation supplémentaire du marché. Les conclusions concernant l'efficacité des stratégies de protection des cultures sont cependant assujetties à l'hypothèse de la présence de bio-agresseurs et des conditions favorables à leur développement au moment de l'expérimentation. Si la pression de ravageurs est facilement estimable par leur observation sur les plantes, ce n'est pas le cas pour les pathogènes dont la présence ne se traduit pas toujours par le développement de symptômes. La caractérisation de la pression de pathogènes durant l'expérimentation, qui requiert des moyens techniques importants, pourrait assoir davantage les résultats des expérimentations systémiques. La poursuite de la collaboration entre l'entreprise Green Produce et l'unité PSH de l'INRA d'Avignon, initiée par cette thèse, devrait également permettre de valider ces résultats dans un plus grand nombre de situations pédo-climatiques.

L'évaluation globale dans l'approche intégrative a également soulevé de nouvelles interrogations quant à la compatibilité entre les techniques alternatives et aux conséquences économiques et environnementales d'une réduction d'usage de pesticides, qui pourraient faire l'objet d'expérimentations factorielles. Par exemple, la quantité de résidus de pesticides détectés à la récolte n'était pas forcement lié à la quantité de pesticides appliqués pendant la culture et les facteurs de cette variabilité doivent être identifiés pour améliorer cette performance. Les deux approches que nous avons utilisées sont en fait tout à fait complémentaires, s'alimentent entre elles, et permettent d'inscrire ces travaux de recherche dans une démarche d'amélioration continue de l'efficacité et des performances des stratégies alternatives de gestion des bio-agresseurs de la laitue.

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Annexes

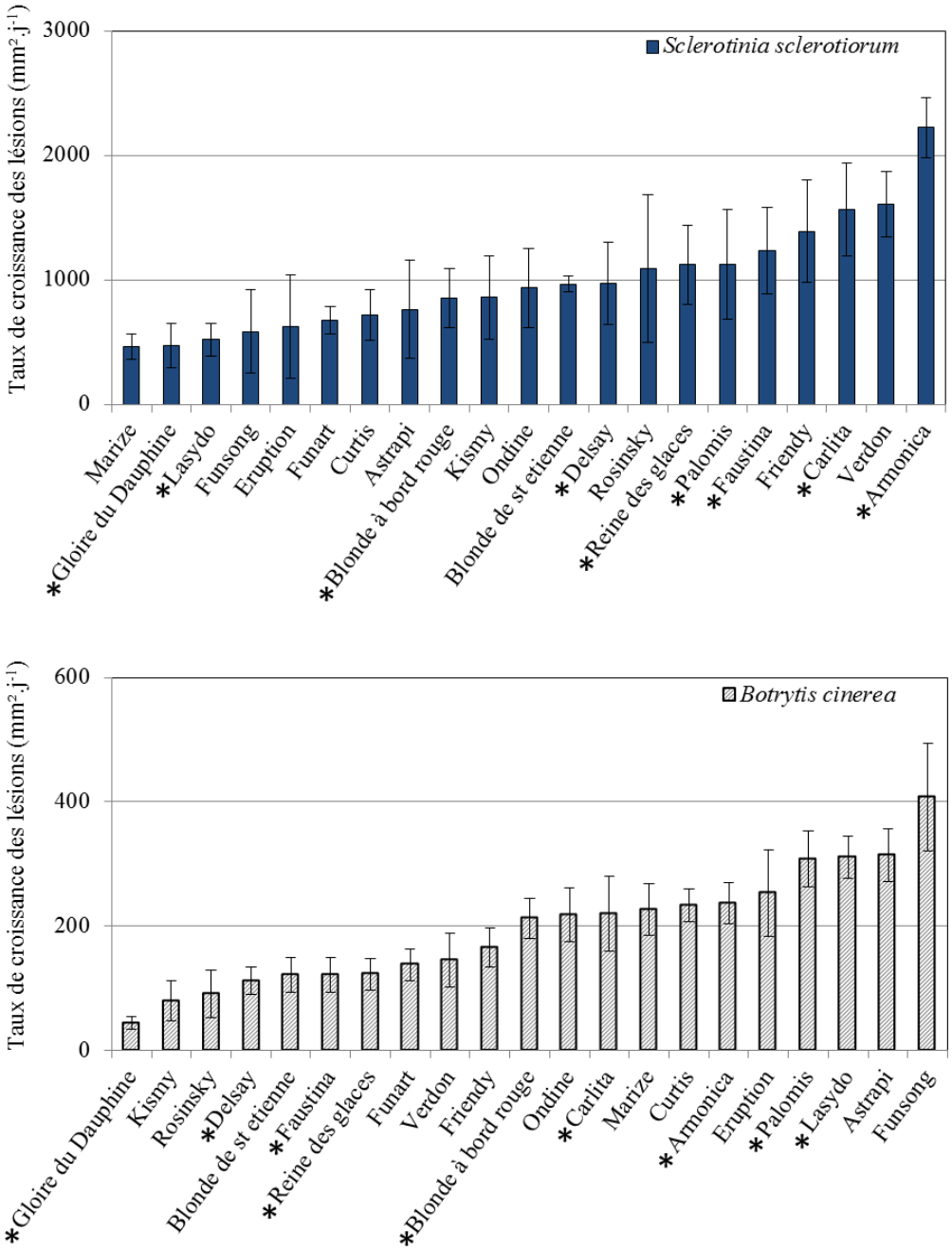
Annexe 1 - Criblage de 21 accessions de laitue pour leur sensibilité à *Sclerotinia sclerotiorum* et *Botrytis cinerea*, Résultats des tests préliminaires.

L'objectif de ce test était d'examiner la gamme de variabilité de réponse à l'inoculation de *B. cinerea* (souche BC1) et *S. sclerotiorum* (souche SS1) sur feuilles détachées de 21 accessions. La méthode d'inoculation est décrite dans le chapitre 3. Les 21 accessions sont les 9 utilisées dans le chapitre 3, plus les accessions présentées dans le tableau ci-dessous.

Type	Accessions	Origine (année d'enregistrement, utilisation)
Batavia rouge	Rosinsky	Rijk Zwaan (2006, plein champ et tunnel)
Batavia verte	Astrapi	Gautier Semences (2013, tunnel)
	Blonde de St Etienne	Public cultivar
	Curtis	Gautier Semences (2013, tunnel)
	Friendy	Enza Zaden (2012, tunnel)
	Funarte	Syngenta seed (2006, tunnel)
	Funsong	Syngenta seed (2009, tunnel)
	Kismy	Enza Zaden (2009, tunne)
	Marize	Enza Zaden (2012, tunnel)
	Ondine	INRA (1976, tunnel)
	Verdon	Gautier Semences (2008, tunnel)
'Grasse' rouge	Eruption	Enza Zaden (2000)

Type horticultural et origine des accessions utilisées dans le test préliminaire.

Le taux de croissance des lésions a varié entre 44 $mm^2.jour^{-1}$ et 400 $mm^2.jour^{-1}$ pour *B. cinerea* et entre 464 $mm^2.jour^{-1}$ et 2227 $mm^2.jour^{-1}$ pour *S. sclerotiorum*. La couleur des feuilles de laitue (rouge ou verte) n'a pas eu d'impact significatif sur la sensibilité à *B. cinerea* (F= 2.2142 ; 1 df ; P= 0.1404) et *S. sclerotiorum* (F= 0.2031 ; 1 df ; P= 0.6534). Les 9 accessions utilisées dans le chapitre 3 ont été choisies pour représenter la gamme de variabilité observée dans ce test préliminaire.



Taux de croissances des lésions (moyenne pour 5 plantes) causées par *S. sclerotiorum* et *B. cinerea*. Les accessions précédées du symbole ∗ ont été utilisées dans l'expérimentation présentée dans le chapitre 3.

Annexe 2 - Caractéristiques des pesticides (fongicides et insecticides) utilisés dans l'expérimentation systémique.

Cibles	Produit phytosanitaire	DAR	Dose	Formulation	Substance active	Composition (%)	Famille chimique	Type d'action	Mode d'action	LMR (mg/kg)
Bremia lactucae	Infinito	21	1,6 l/ha	suspension concentrée	Propamocarbe	55,50	carbamates	systémique	Altération des membranes cellulaires, action sur la croissance mycélienne, la production de sporanges et germination des oospores des phycomycètes	50
					Fluopicolide	5,30	acylpicolides	systémique	Délocalisation des protéines reliant la membrane du cytosquelette	8
	Sygan	28	2,5 kg/ha	Granules dispersibles	Cymoxanil	4,80	acétamides	systémique local	Inhibition de la biosynthèse des acides nucléiques des lipides et des acides aminés et modification de la perméabilité cellulaire.	0,2
					Folpel	20,00	phtalimides	contact	Inhibition de la germination des spores	2
					Mancozèbe	20,00	dithiocarbamates	contact	Inhibition de la germination des spores	5
Sclerotinia spp. et Botrytis cinerea	Signum	14	1,5 kg/ha	Granules dispersibles	Pyraclostrobine	6,70	strobilurines	translaminaire	Inhibition de la germination des spores	2
					Boscalid	26,70	carboxamides	translaminaire	Action au niveau de la respiration et de la production d'énergie. Inhibition de la succinate déshydrogénase (SDH, complexe II) dans la chaîne de transport mitochondrial des électrons.	10
	Switch	14	0,6 kg/ha	Granules dispersibles	Cyprodinyl	37,50	anilinopyrimidines	systémique	Inhibition de l'élongation des tubes germinatifs et des hyphes mycéliens	10
					Fludioxonil	25,00	phénylpyrroles	contact	Perturbation des échanges membranaires en jouant sur la pression osmotique	10
	Rovral	14	1 kg/ha	Granules dispersibles	Iprodione	75,00	dicarboximides	systémique local	Inhibition de la germination des spores et de l'élongation des hyphes mycéliens	10
Pucerons	Pirimor G	14	0,75 kg/ha	Granules dispersibles	pirimicarb	50,00	carbamates	translaminaire	Inhibition de l'activité de l'Acetylcholinesterase	5
	Suprême	7	0,25 kg/ha	Poudre soluble	Acetamipride	20,00	chloronicotiniles	systémique	Antagoniste de l'Acétylcholine	5
	Movento	7	0,75 l/ha	Suspension concentrée	Spirotetramat	9,30	kétoénoles	systémique	Inhibition de la biosynthèse des lipides	7